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Conservation of South Florida Orchid Mycorrhizal Fungi

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CONSERVATION OF SOUTH FLORIDA ORCHID MYCORRHIZAL FUNGI

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in

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by

Ellen Garcia

2021

To: Dean Michael R. Heithaus
    College of Arts, Sciences and Education

This thesis, written by Ellen Garcia, and entitled Conservation of South Florida Orchid Mycorrhizal Fungi, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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Date of Defense: July 1, 2021

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Florida International University, 2021

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ABSTRACT OF THE THESIS

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Professor Amir Khoddamzadeh, Major Professor

Starting in the late 1800's orchids were heavily poached, leaving many species to reach critically low numbers. Coupled with habitat loss and urbanization many orchid populations were extirpated in southern Florida. Due to lack of endosperm, orchid seeds are reliant on specific mycorrhizal fungi to obtain nutrients to enable growth and development resulting in very low germination rates in nature. The obligate relationship on mycorrhizal fungi complicates orchid re-establishment. The research project aims are (1) to evaluate the correlation between phenotypic traits and optimal growing conditions in various micro-climate, and (2) to assess the range of mycorrhizal diversity in urban and botanic garden settings. The native orchid *Encyclia tampensis*, was sampled from naturally occurring and lab propagated orchids at Fairchild Tropical Botanic Garden (FTBG), Naples Botanical Garden (NBG) and Downtown Doral Park (DDP) on oak, cypress, and palm trees. Phenotypic measurements (host tree, chlorophyll content, light intensity, height on the tree, number of leaves, root and shoot lengths) and root sampling (fungal isolations, DNA identification) were conducted. Increased SPAD and NDVI values measuring chlorophyll content as a plant health parameter were observed at FTBG, while DDP and

NBG had comparable values indicating the suitability of botanic gardens and urban spaces as orchid reintroduction sites. Across all 3 sites and host trees, the beneficial fungi *Tulasnella* was recovered within two distinct clades. The results of this study will help maximize current conservation and reintroduction programs in orchids within urban areas and botanical gardens for best management practices.

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1. INTRODUCTION

Orchidaceae is one of the largest and most diverse families of flowering plants and contains approximately 25,000 species and 880+ genera worldwide (Givnish et al., 2015). They are also well known for having a wide range of uses such as: ornamental, medicinal, and cultural values (Vendrame and Khoddamzadeh, 2016). Habitat loss, environmental degradation, over-collection, and periodic freezes has led many orchid species to reach critically low population numbers. It has been approximated that more than 10% of all orchid species worldwide are threatened (Yang et al., 2017). Furthermore, for species that typically grow as epiphytes on a host tree, only seeds that land on trees containing the compatible mycorrhizal fungi will germinate and develop into seedlings. This can further decrease the already small chance of seeds to become established and develop (Rasmussen et al., 2015). Therefore, it is important to understand the impacts of various abiotic and biotic factors on the success of orchid growth and how these factors affect the overall success of survivorship. These factors include: host tree species, light intensity, and mycorrhizal fungi (Yang et al., 2017).

The importance of orchid conservation is not only intrinsic but also for their role as potential indicators of changes in ecosystem health. Several biotas depend on epiphytes for their various needs, either for moisture, nectar, pollen, fruits and seeds. Epiphytes also serve as water storage, provide supplement of secondary metabolites, nesting and breeding spaces. In forestry management, they have been used to indicate increasing levels of atmospheric pollution. Epiphytic communities are sensitive to changes in atmosphere and climate and are expected to be the first communities to show drastic responses to impending climate change (Shashidhar and Kumar, 2009).

1

Even with this strong sensitivity to environmental changes, orchids have been observed to colonize urban and non-natural landscapes (Yam et al., 2011, Bhatt et al., 2015). This suggests the potential of using urban landscapes and botanic gardens for orchid refuges. To be able to improve the success of conservation programs in urban and botanical garden settings, comprehensive data collection is imperative (Izuddin et al., 2019).

Epiphytic orchids rely on the microbiome of the bark on certain host trees for nutrient uptake. The mycorrhizal fungi that associates with orchids within the root cortex can be identified using molecular based techniques to give insight on mycorrhizal diversity. This novel research can contribute very important knowledge that opens the door to new questions to be asked such as: what are the factors impacting orchid diversity and abundance? To what degree do abiotic and biotic factors influence mycorrhizal diversity and therefore orchid establishment, health, and growth? This new frontier in orchid biology is shedding light and guiding the way for future scientists to expand knowledge, and it is uncovering the way in which the collective microbiome of a plant plays a key role in global ecosystems and species diversity (Herrera et al., 2019) and the way in which microorganisms have co-evolved with plants to guide the range of expansion and biodiversity in nature. Studying this topic provides evidence for the importance of conserving nature for species with a narrow niche range. This is especially critical in the face of climate change, since many vulnerable rare species are sensitive to environmental conditions.

This research is focused specifically on the abiotic and biotic factors effecting the commercially exploited orchid, *Encyclia tampensis,* on varying host trees in south Florida

urban landscapes. Recently, through the Million Orchid Project conservation program, lab grown orchids have been reintroduced into urban landscapes. However, sampling had not been previously done to determine the presence of compatible mycorrhizal fungi in lab propagated orchid roots within these urban environments. Mycorrhizal fungi are required for the germination of orchid seeds, so the isolation and identification of mycorrhizal fungi is critical for creating the best conservation practices. It is vital to understand which mycorrhizal species they might be interacting with and to what extent, as well as how this varies across different habitats. In this study, *E. tampensis* was sampled at Fairchild Tropical Botanic Garden, Naples Botanic Garden and Downtown Doral Park on oak, cypress and palm trees. The site locations and host tree species will result in significant differences in plant growth characteristics (shoot length, root length, number of leaves), plant health parameters (leaf chlorophyll content) and mycorrhizal diversity in *E. tampensis.*

2. LITERATURE REVIEW

2.1 Orchid Diversity

Orchid seeds are extremely small, with a length of 0.25–1.20 mm and width of 0.09–0.27 mm, and each capsule may contain 1300–4,000,000 seeds (Arditti 1967, Knudson 1922). Due to lack of endosperm, orchid's seeds are reliant on specific mycorrhizal fungi to obtain nutrients to enable growth and development resulting in very low germination rates in nature. Loss of hardwood hammock and swamp habitat has negatively impacted understory plant populations, such as native orchids in Florida (Sprott and Mazzotti 2001). Many of the orchids found there are listed as either threatened or endangered on Florida's Regulated Plant Index (Coile and Garland 2003). In Florida there are approximately 100 native orchid species, many of which are rare and endangered (IRC, Luer 1972). *Encyclia tampensis* (Lindl.) Small (Tampa Butterfly Orchid), an epiphytic orchid that is listed as commercially exploited, inhabits several swamp locations within the Florida Panther National Wildlife Refuge (FPNWR). *E. tampensis* commonly grows in freshwater wetland forests, which alternate between having a dry forest floor, and standing water during the rainy season (approximately May–October) (Ray et al., 2020).

2.2 Effects of Environmental Factors (biotic and abiotic) and Climate Change on Orchid Mycorrhiza

Over the next century, climate changes are expected to occur globally and have an effect on Research has shown the rapid change of climate occurring globally over the next century and inevitably causes changes in habitat functions. With continually

increasing CO_2 concentrations and global surface temperatures increasing between 1.8 to 3.6 C by the year 2100, there is a need for protecting habitats and mitigating biodiversity loss (IPCC Climate Change, 2007). This is especially important for environmentally sensitive orchid species, as beneficial plant-associated microorganisms that stimulate growth and enhance the resistance to disease and abiotic stress may be affected. The correlation between elevated CO_2, drought, and warming and the response of microorganisms to these changes has been explored by many researchers. In a review of 135 studies investigating the effect of climate change on beneficial microorganisms and their effect on plant growth, the plants with beneficial symbiotic relationships showed positive traits in response to elevated CO_2 (Compant et al., 2010). This shows importance of the benefits available within plant-microorganism relationships in response to changes in climate and in the atmosphere.

Climate has an effect on both fungal and orchid distribution. A study in annual abundance of *Corallorhiza odontorhiza* was statistically related to growing season precipitation and winter temperature (McCormick et al., 2009). The distribution of orchids within natural areas ben shown in several cases to be related to the abundance and distribution of appropriate host fungi (Pecoraro et al., 2018; Djordjević and Tsiftsis, 2020; Kaur et al., 2020). This indicates that mycorrhizal fungi necessary for orchid reproduction is likely correlated with the micro-climate of the area. For example, the beneficial fungi species *Tomentella* was most abundant in areas with high orchid abundance, and was less in areas with few or no orchids. This was the first indication that the relationship between plants and mycorrhizal fungi is influenced by the abundance of

fungi that support orchid growth as well as specific fungal types that are particularly

suitable for facilitating orchid growth (McCormick et al., 2009).

2.3 Mycorrhizal Diversity

Most land plants have an association with mycorrhizal fungi. This interaction can

have an effect on the survival and distribution of a species and in the case of orchids, they

depend on the distribution of suitable fungi within its habitat (Dickie et al., 2017;

Downing et al., 2020) which could also affect rarity (Swarts et al., 2010; Phillips et al.,

2011). The fungus enters the root cortex and the hyphae grows and expands to coil and

produce pelotons for carbon uptake by the orchid by molecular mechanisms that are

currently being researched. All orchids are fully dependent on fungi for seed germination

and many also for subsequent growth and survival. Generating orchid seedlings

symbiotically for reintroduction has been shown to increase survival probability when

compared to asymbiotically produced seedlings (Yang et al., 2017). Yet these techniques

are much more difficult and not cost effective in most cases.

At the ecosystem level, most species interact with a wide range of partner species.

The role of evolution in constituting these interactions is unknown thus far, mostly

because it is difficult to compare the extent and range of the relationships. The specificity

is based on the degree of specialization that occurs between the interacting species and on

the opportunity for them to interact. This is based on species richness and phylogenetic

diversity. According to Downing et al. (2019), there are three modes of interaction

specificity: (1) assemblage specialization, where a species specializes on a particular host

species that contributes unique resources, (2) apparent generalism, which is the case

when a species specialized on one or few host species that contribute redundant resources and (3) true generalism, where a species associates with a wide range of hosts that overlap in functionality and are geographically interchangeable, allowing for host switching.

Specificity has been used as a measure of specialization, however it is really determined by the biological compatibility of partners and their biogeographic limits, and this depends on environmental factors. The extent of the evolutionary specialization of a host species depends on the degree to which the interaction with that host species allows for a fulfillment of its niche. Mycorrhizal fungi has been found to dictate the abundance, spatial distribution and coexistence of orchids in their natural communities (McCormick et al., 2016).

Beneficial orchid mycorrhizal fungi (OMF) isolations are critical to improving the survival rate of endangered species grown from tissue culture or transplanted. In nature, a species ability to expand its population and increase its range is heavily dependent on the biotic interactions present within the ecosystem. If the species has a narrow specificity in terms of biotic factors, this would limit their ability to spread. Below ground biotic interactions have a major role in influencing orchid range through specificity of mycorrhizal fungi and pathogenic species as well.

Tulasnella fungi have been seen as an especially important factor in the growth of multiple orchid species, where some strains may be more effective than others in both compatibility with a wide range of species as well as inducing seed germination and supporting subsequent growth (Suarez et al., 2006, Fuji et al., 2020, Silva et al., 2020, Wang et al., 2021). The research presented by Downing et al. (2020) expresses the

hypothesis that generalized mycorrhizal interactions along with the ecological concept of enemy release plays a major role in driving the range expansions of orchids found in southern Florida. This is important because of the effect of complex interactions between orchids and fungi and how this guides biodiversity. This suggests that there are large fungal network intricacies involved in the rarity of certain plants.

Orchid mycorrhizal diversity has been found to associate with many species of basidiomycetes or ascomycetes. Examples of common basidiomycetes found in orchid roots are: *Ceratobasidiaceae*, *Tullasnellaceae*, and *Sebacinaceae* (Pecoraro et al., 2018). These are known to associate with orchids to provide nutrients and enable germination. Meanwhile, ascomycetes are typically found to be associating with orchids in the form of plant pathogens. Many greenhouse-grown orchids have been found infested by leaf spot disease or ringworm disease caused by *Phyllostictina pyriformis* Cash and Watson, black spot disease caused by *Alternaria alternate* and *Drechslera,* anthracnose disease caused by *Colletotrichum gloeosporioides,* dry rot or wilt disease caused by *Fusarium oxysporum Fmoniliforme*, soft rot disease caused by *Erwinia carotovora*, yellow leaf spots disease caused by *Pseudocercospora dendrobii* and dry rot disease caused by *Sclerotium rolfsii*. However, one study found *Fusarium oxysporum* KB-3 to promote germination and growth in *Bletilla striata* (Jiang, 2019). After correctly identifying the infestation, the correct amount of the chemical must be precisely sprayed at the designated areas to determine the application's efficiency as well as its production cost. Incorrect application of chemicals would not only result in economic losses, but also cause environmental damage (Samseemoung et al., 2017).

2.4 Host Trees

Trees in urban environments can provide potential habitat for epiphytic plants to enhance the local diversity. The extent and presence of compatible OMF on urban trees has not been researched to a wide extent (Downing et al. 2020). In a study by Izuddin, et al. (2019), the suitability of urban trees to support OMF and native epiphytic orchids was assessed by means of data collection on tree micro-climate. This was done using high-throughput sequencing of orchid root samples to detect OMF and data on the porosity and roughness of host tree bark (Izzudin et al., 2019). Among the suitable host trees, there may be one particular species that is especially suitable for promoting orchid growth. This was seen in a study focused on the green fly orchid which are typically found in rare inland forests in Georgia. In their natural habitat 54% of orchids surveyed were identified on Southern magnolia, 31% on live oak and varying minimal percentages on 5 other tree species (Bergstrom and Carter, 2008). The research shows that certain tree species provide more favorable microclimates in terms of light intensity, humidity, precipitation, temperature. Host trees with the most orchids were also dependent on tree size and age (Bergstrom and Carter, 2008; Adhikary et al., 2012). In particular, mature, tall, and large host trees (with reference to diameter) have been shown to have an important role in the structure of the orchid-host tree network nestedness and robust patterns when modeled and therefore should be paid special attention to conserve the orchid communities and the organisms associated with them (Zotarelli et al., 2018; Abe et al., 2018).

The bark microenvironment of the phorophyte plays an important role in supporting orchid abundance and richness, this is dependent on the host characteristics

topology (bark porosity) and water storage capacity and the preference of orchids which in turn affects orchid diversity and distribution worldwide (Timisina et al., 2016; Zarate-Garcia et al., 2020;). In tropical dry forests of Yucatan, Mexico, five epiphytic orchid species were compared in two dry forest fragments differing in tree composition, stature and rainfall regime, where the microclimate conditions were assessed and showed higher orchid abundance in the low precipitation site, in middle canopy stratum and 90% of orchids grew on a single host tree. Although the bark roughness and area of the substrate of the host were important factors, this did not explain the preference and may be due to other factors such as presence of mycorrhizal fungi (Rosa-Manzano et al., 2014).

Many studies have focused on the correlation between orchids and host characteristics as mentioned, however little is known about the differences in mycorrhizal and endophytic fungal communities in epiphytic orchids growing on different host tree species. In a study by Wang et al. (2017), the species richness and diversity of mycorrhizal and endophytic fungal communities isolated from orchid roots were strongly influenced by host tree species. Since orchids have been found to be particularly sensitive to the abiotic and biotic factors, this makes identifying the species-specific niche requirements vital to be identified and then integrated into conservation plans (Izuddin, 2018).

2.5 Growth Monitoring and Plant Health Assessments

A SPAD-502 meter was used to measure chlorophyll content of the leaves as a proxy for nutrient absorption, such as the availability of nitrogen to the plant (Konica Minolta, Ramsey, NJ, USA). The SPAD meter is a non-destructive handheld sensor that

gives a numerical value of the approximate amount of chlorophyll present in a given leaf. The SPAD meter clips onto a single leaf and records light intensity of 650 nm and 940 nm, measuring the absorbance of light emitted where the 650 nm wavelength is correlated to maximum chlorophyll activity while the 940 nm wavelength accounts for other factors including leaf thickness and moisture content. The difference of the two absorbance values for the light emitted at a given wavelength gives the user a value ranging from -9.9 to 199.99 (Perez-Patricio et al., 2018).

Non-destructive optical handheld sensors Soil Plant Analysis Development (SPAD) and GreenSeeker[TM] (Trimble, Sunnyvale, CA, USA) provides an indication of chlorophyll content within green plants material. This allows for better understanding of a plant's overall health, vigor and nutrient content at a given time. The GreenSeeker[TM] device contains two electroluminescent diodes that emit red (660nm) and near infrared (NIR) light (770nm) onto the canopy of the target plant and the reflectance of the light is based on the plants green color that exhibits a low reflectance of red and blue and high reflectance of NIR radiation. Vegetation strongly reflects near infrared light and red light is strongly absorbed. Chlorophyll in the leaves are responsible for the reflectance of the visible red light and scattering NIR. NDVI values are the NIR minus red divided by the NIR plus red. Therefore, the NDVI values are a ratio ranging from 0 (low chlorophyll content) to 1 (substantial chlorophyll content and dense green leaves) (Freidenrich, 2019).

In orchid conservation, a SPAD device can be used to measure chlorophyll content or "greenness" of plants to give an instant read on plant health, diseases, yield limiting deficiencies or fertilizer deficiencies which then lead to yield decrease or plant

mortality in horticultural crops (Dunn et al., 2015; Khoddamzadeh and Dunn, 2016; Freidenreich et al., 2019). A GreenSeeker™ device is another handheld device that is a portable battery-operated NDVI sensor used for monitoring nitrogen levels as well. These optical sensors measure and quantify the variability of the crop and may create a targeted prescription agriculture and best management practices (BMP) in horticultural specialty crops (Dunn et al., 2015; Freidenreich et al., 2019).

In a laboratory setting orchids are grown in a nutrient rich agar-based medium. They are then acclimated in a greenhouse for up to one year and planted into urban landscapes. In a nursery setting, orchids are typically fertilized weekly at varying nutrient levels depending on their different stages of growth whether they are in a nursery, plantation, in the flowering stage or at the flower cutting stage. Once they are reintroduced into urban areas, they are susceptible to disease and insect pest infestation.

The SPAD and GreenSeeker™ devices have been employed extensively in research and agriculture, with a range of different plant species for growth monitoring and plant health assesments (Ling et al., 2017; Khoddamzadeh and Dunn, 2016). However, it has not been widely used in relation to orchid conservation. Using SPAD and GreenSeeker™ can show promising results in determining fertilizer requirements of nursery grown plants (Freidenrich et al., 2019).

2.6 Urban Ecology and Reintroductions: Acclimatization to Microclimates and Urban Environments/Gardens

Habitat loss due to urbanization and human population growth puts increasing pressure on biodiversity worldwide. As population sizes continue to rise globally, there

is a tendency towards creating more residences in urban areas and this continues to cause biodiversity loss. This urbanization leads to habitat degradation, and fragmentation leading to demographic or genetic isolation and species extirpation. With this increased loss, conservation horticulture management practices in urban spaces are imperative to contribute to biodiversity conservation. Therefore, the conservation of orchid populations may depend on the establishment of lab propagated orchids to be transferred to the field sites to help sustain threatened populations and encourage natural recruitment (Jacquemyn et al., 2014).

Little is known about the biotic and abiotic conditions influencing establishment and mortality of transplanted orchids. A study done in Australia showed overall survival rates of (Plantlests) to be 49% for *Microtis media* and 21% for *Caladenia arenicola* (Scade et al., 2006). These strategies allow for the integration of native species back into urban areas as land use has an effect on richness and abundance of orchids that is dependent on various environmental conditions such as surrounding vegetation (Alzate et al., 2019). Habitat fragmentation can significantly affect plant reproductive success and population viability and impact plant-pollinator mutualisms as well. One study investigating this orchid-pollinator relationship found that over 40% bare ground cover resulted in reproductive failure (Newman et al., 2013). Roadsides have also been found to be a suitable habitat for orchids that grow in both forested and grassland habitats (Fekete et al., 2020), where differences in the environment and vegetation communities affects what species of orchid is found there (Landi et al., 2008). For these conservation strategies to be successful, there is an urgent need for assessing the capacity of these spaces to support threatened species.

3. METHODOLOGY

3.1 Plant Species

Encyclia tampensis is an epiphytic monocot that is perennial and has native ranges from Florida to the Bahamas. The global status is apparently secure, the state of Florida status is commercially exploited and is regionally secure (IRC, 2020).

Figure 1 *Encyclia tampensis* on *Quercus germinata* host tree at Naples Botanical Garden.
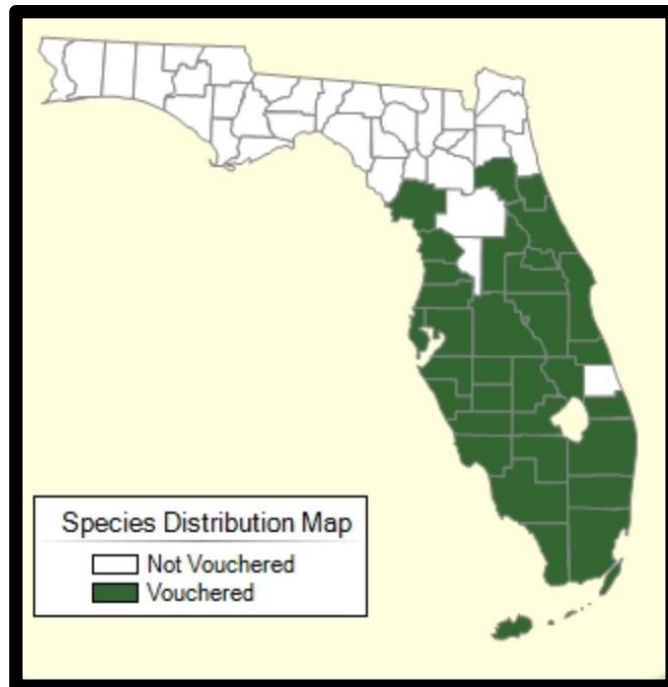
Figure 2 Map of the native range of *Encyclia tampensis* in Florida, USA (IRC, 2020).

3.2 Site Description and Experimental Design

The study was conducted at Fairchild Tropical Botanic Garden, Downtown Doral Park and Naples Botanical Garden. Sampling was done of *Encyclia tampensis* orchids on 3 varying host trees: Oak (*Quercus virginiana* at FTBG and *Quercus germinata* at NBG), Palm (*Roystonea regia*), and Cypress (*Taxodium ascendens*). In total, 25 individual plants were sampled. Phenotypic measurements were taken and a 1-inch root cutting was collected from each plant. At Fairchild Tropical Botanic Garden, 10 plants were samples, 5 on live oak (naturally occurring) and 5 on palm trees (reintroduced and grown in a lab setting). Located in Downtown Doral Park within an urban area, 10 plants were sampled, 5 on live oak and 5 plants on dwarf cypress trees. At Naples Botanical Garden, 5 plants were sampled, all on live oak and naturally occurring. The habitat was within a pine rockland conservation area.

Figure 3 Lab propagated outplants of orchid seedlings grown asymbiotically in E-medium at Fairchild Tropical Botanic Garden.
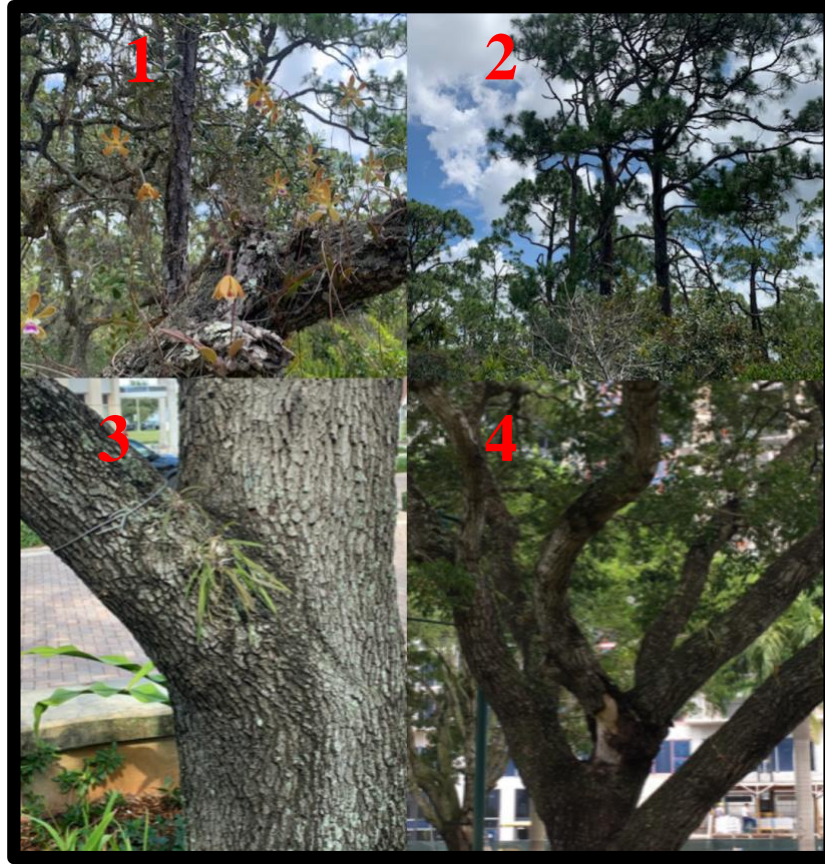
Figure 4 Photos taken at sampling sites. (1) *Encyclia tampensis* on *Quercus Germinata* at Naples Botanic Garden, (2) Pine Rockland habitat at Naples Botanic Garden where naturally occurring orchids were sampled from, (3) *Encyclia tampensis* outplant attached to *Quercus virginiana* by means of zip tie, and (4) *Quercus virginiana* host tree at Downtown Doral Park.

3.3 Field Sampling and Data Collection Methods

Data was collected on the host tree species, chlorophyll content using SPAD and GreenSeeker™ handheld devices, light intensity, height and orientation on the tree, number of leaves, root and shoot lengths. Using the SPAD device, 3 mature leaves were selected and random from opposing sides of the plant and the measurements were averaged. The SPAD electrode was placed in the central region of each leaf measured. Measurements are proportional to the amount of chlorophyll present in the leaf. In order to convert these values into absolute units of chlorophyll concentration, calibration curves

must be derived and utilized. The equation shown below can be used to convert SPAD into total chlorophyll per unit in leaf area (Ling, Huang, and Jarvis, 2017)

For the Green seeker device to obtain the NDVI values, the measurements were taken 3 times in consideration of the variation of foliage on the canopy of each plant and the mean was taken of the 3 measurements when analyzing the data. The plants green leaves were targeted to minimize background noise when utilizing the device and held about 45cm above the sample plant for each reading and ensures the field of view for the center is approximately 11.85 cm in diameter (Samseemoung et al., 2017).

$$NDVI = \frac{(NIR - Red)}{(NIR + Red)}$$

NIR – reflection in the near-infrared spectrum
RED – reflection in the red range of the spectrum

3.4 Statistical Analysis

All statistical analysis was performed at $P < 0.05$ level using SPSS statistical software (Version 22.0., IBM Corp., Armonk, NY, USA). Data trends will be compared using one-way ANOVA and correlation tests, significant differences between variables will be found using post-hoc least significant differences (LSD) method. This data will be used to assess best management practices and optimal growing conditions for planting orchids from the lab into their native ranges.

 3.5 Laboratory Methods

3.5.1 Media Preparation, Isolation of Mycorrhizal Pelotons, and Culturing Media

These protocols were used to culture and genetically identify mycorrhizal fungi found in the roots of *Encyclia tampensis.* Preparation for orchid mycorrhizal fungi was

done by creating E-medium: BS and NBS. The media contains glucose, ammonium tartrate, KH$_2$PO4, MgSO$_4$*7H$_2$O, CaCl$_2$*2H$_2$O, yeast extract, ferric citrate, MnSO$_4$*1H$_2$0, ZnSO$_4$*7H$_2$O, agar and 1L DI water with NBS (Novobiocin antibiotic) in the initial isolation of mycorrhizal fungi. For subculturing, the same media was used without containing the Novobiocin antibiotic.

Samples were collected across 3 sites: Fairchild Tropical Botanic Garden, Downtown Doral Park and Naples Botanic Garden. Once the samples were collected, they were washed with DI water to remove organic material and debris from the root surface. Roots were moved to the laminar flow cabinet using sterile equipment to isolate mycorrhizal fungi pelotons. Tissues were rinsed in 3 sterile water tubes using a serial dilution technique. Each tube with the root sample was placed in water for 3 minutes and then vortexed for 1 min to continue removing debris from the root surface. The approximately one-inch root cutting was placed in a petri dish and sterile DI water was added to cover the root and analyzed under a dissecting microscope. A sterile scalpel was used to scrape the outer layer of the orchid tissue while using forceps to hold the root cutting in place. Fungal pelotons are visible under the microscope and appear as small, white or cream, oblong to ovoid, granular structures and begin to disperse in the sterile water droplet once the outer layer is scraped.

The pelotons are also visible as hyphae coils within the root cortex. The next step was to complete a serial dilution of pelotons. Using a pipette 5 water droplets of 400 ul were placed in one petri dish. Using a pipette, the individual pelotons moved from one water droplet to another at 1 ul increments. Pelotons were collected from the isolation droplet in as little water as possible and ejected into the fresh droplet. The peloton

transfer procedure was repeated for each droplet with a fresh pipette in between droplet

changes. Some pelotons are lost in the water changes, but a large percentage is recovered.

Each root cutting was stored in Eppendorf tubes to be used for DNA extraction

procedures. Procedures used are from Swarts et al. (2010) with minor modifications.
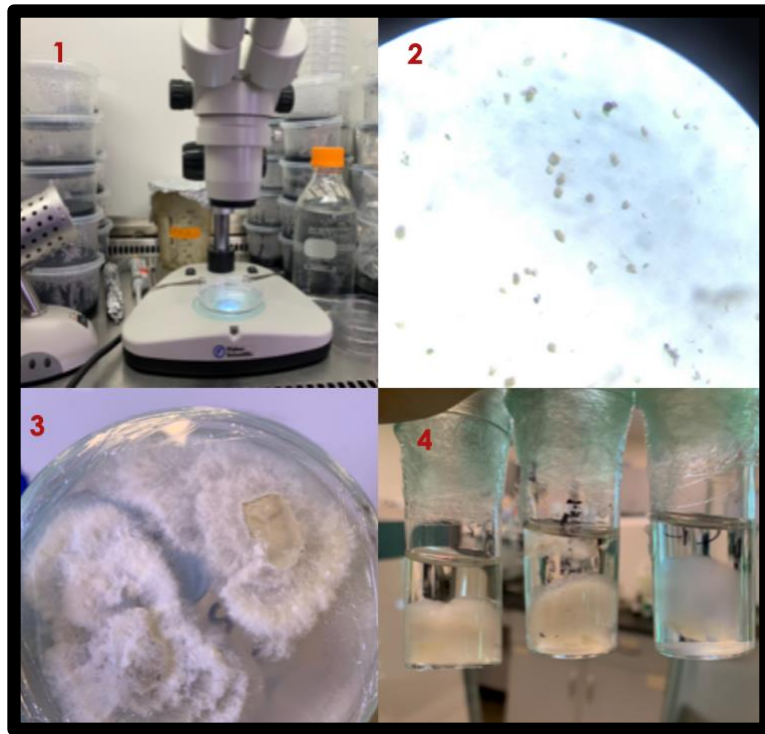
Figure 5 Photos taken at Fairchild Tropical Botanic Garden Micropropagation lab. (1) dissecting microscope, (2) mycorrhizal pelotons as seen through the dissecting microscope, (3) mycorrhizal fungal culture, (4) mycorrhizal fungi liquid culture.

3.5.2 DNA Extraction, PCR, and Sequencing

Root cutting and liquid culture sample DNA extraction procedure were carried out

using the DNeasy Plant Handbook, purification of total DNA from plant tissue mini

protocol using the DNeasy Plant Mini Kit. PCR amplification was conducted for

selective screening of mycorrhizal fungi. Three fungal primers and specific PCR cycle

temperature and timings were used: ITS1F, ITS4, ITS1OF1 and 2, ITS4OF, and ITS5tul.

Within these 3 primer sets, one set is a universal primer for genetic kingdom fungi, the

second set is used to screen for known orchid fungi groups, and the third is used to selectively amplify *Tulasnella* fungal species. Primer mix consists of 6.25 ul Master Mix, 2.5 ul TBT buffer, 1.75 ul DD H2O, 0.75 ul reverse primer, 0.75 forward primer, and 1.0 ul root or liquid culture DNA added.

To prepare the PCR product for sequencing, a PCR clean up procedure and sequencing reaction was done. The PCR cleanup is done to remove enzymes, nucleotides, primers and buffers. PCR cleanup was done using 2ul ExoSAP-IT mix and 5 ul of PCR output and run on a thermocycler at 37°C for 15 min and then 80°C for another 15 min. The sequencing reaction is done to generate longer and higher quality reads by sequencing only primer at a time (forward or reverse primer for each of the 3 sets). This is done using the BigDye Terminator v3.1 Cycle Sequencing kit. This consists of 2 ul of DNA added to a PCR tube and a master mix of: 5.5 ul DD H$_2$O, 2.0 ul BigDye Buffer, 0.5 ul of 1.87 uM forward or reverse primer, 1.0 ul of Big Dye Terminator and 1.0 ul of PCR product post PCR clean up (Downing et al., 2020).

3.5.3 Molecular Analysis/Phylogenetic Tree Assembly

Sequences obtained via sanger sequencing were run on NCBI BLAST: Basic Local Alignment search tool to find the known fungal sequences with the highest similarities greater than 97%. All sequences were manually trimmed and optimized and aligned using the MAFFT plugin. PHYML plug-in was used to create estimations of phylogenies by generating consensus trees using neighbor-joining, and maximum likelihood with 10 random addition replicates using Geneious software (Kearse et al., 2012).

4. RESULTS

4.1 Host Tree ANOVA Analysis of *Encyclia tampensis* Growth Properties and Plant Health Parameters

Plant growth on varying host trees was evaluated by collecting data on orchid growth properties such as height on the tree, shoot length, root length, and number of leaves. Plant health parameters were designated as the chlorophyll content obtained using SPAD and GreenSeeker[TM] handheld devices and the amount of OTUs of basidiomycetes and ascomycetes recovered per sample. None of the different host trees were significantly different from each other ($p > 0.05$), when it came to the orchid growth properties (Table 1). The greatest mean heights on the tree were observed in cypress trees ($176.60 \pm 11.28a$), followed by Oak ($143.90 \pm 73.36a$), and palm ($154.38 \pm 10.39a$). Orchids on palm exhibited the highest mean shoot length ($12.65 \pm 2.53a$), Cypress trees contained the highest root length ($21.00 \pm 6.07a$) as well as the greatest mean number of leaves ($16.40 \pm 9.71a$). Sampled on oak showed the lowest mean shoot length (17.43 ± 15.10) and lowest mean number of leaves ($12.33 \pm 7.29a$).

Table 1 Effect of host tree on orchid growth properties.

Host Trees	Height on the Tree (cm)	Shoot Length (cm)	Root Length (cm)	# of Leaves
Palm	$154.38 \pm 10.39a$	$12.65 \pm 2.53a$	$18.7 \pm 10.10a$	$15.20 \pm 11.19a$
Oak	$143.90 \pm 73.36a$	$10.94 \pm 5.34a$	$17.43 \pm 15.10a$	$12.33 \pm 7.29a$
Cypress	$176.60 \pm 11.28a$	$7.60 \pm 1.64a$	$21.00 \pm 6.07a$	$16.40 \pm 9.71a$

Independent variable is characterized as the host tree the orchid was sampled on (Palm at FTBG n=5, Oak n=5 at FTBG, n=5 at DDP and n=5 at NBG, and Cypress n=5 at DDP). Values are expressed as Mean ± Standard Deviation. One-way ANOVAs were performed

on the mean value of the factor evaluated. Means within a column followed by the same letter are not significantly different at p ≤ 0.0500. Significantly different treatments were highlighted by adding a letter next to the mean value.

Additional parameters measured such as SPAD values, NDVI values, basidiomycetes and ascomycetes are shown in Table 2. Orchids placed on palm had the highest average values for both SPAD and NDVI values at (56.18 ± 11.70) and (0.62 ± 0.06), respectively. Cypress trees had the lowest SPAD values (33.90 ± 7.73). When it came to the NDVI values however, oak trees showed the lowest mean value (0.51 ± 0.10). NDVI values (F=5.8676, DF=2, P ≤ 0.05) were significantly greater in orchids on palm trees compared to cypress and oak trees (Table 2).

Table 2 Effect of host tree on orchid plant health parameters.

Host Trees	SPAD values	NDVI values	Basidiomycetes	Ascomycetes
Palm	56.18 ± 11.70a	0.62 ± 0.06a	2.00 ± 1.73a	0 ± 0b
Oak	45.80 ± 16.34ab	0.51 ± 0.10b	1.40 ± 1.30a	0.53 ± 0.83ab
Cypress	33.90 ± 7.73 b	0.42 ± 0.07b	0.80 ± 0.45a	1.20 ± 1.10a

Independent variable is characterized as the host tree the orchid was sampled on (Palm at FTBG n=5, Oak n=5 at FTBG, n=5 at DDP and n=5 at NBG, and Cypress n=5 at DDP). Values are expressed as Mean ± Standard Deviation. One-way ANOVAs were performed on the mean value of the factor evaluated. Means within a column followed by the same letter are not significantly different at p ≤ 0.0500. Significantly different treatments were highlighted by adding a letter next to the mean value.

4.2 Site Location ANOVA Analysis of *Encyclia tampensis* Growth Properties and Plant Health Parameters

The phenotypic data was also analyzed by site, all sampling was done from orchids on oak trees. The site in FTBG was mainly naturally occurring orchids on old

growth and large oak trees, the samples at NBG were naturally occurring orchids on

dwarf sand live oak, and the orchid samples from DDP were outplants grown in a

laboratory setting and placed on smaller and less mature oak trees than those at FTBG

approximately 2 years prior to sampling. Orchids sampled at DDP were the greatest

mean value of height they were placed on the tree after being transplanted from being

grown asymbiotically in a laboratory setting (217.40 ± 55.33) and also had the lowest

mean shoot length (7.50 ± 2.57), with the greatest mean number of leaves (16.60 ±

10.31). Orchids from NBG (120.30 ± 8.14) and FTBG (94.00 ± 71.72) were naturally

occurring on oak trees and had means that were significantly lower from heights on the

trees in DDP (F=7.6607, DF=2 , P ≤ 0.05). Samples from FTBG (17.40 ± 2.75a) had

significantly greater mean shoot length than those at DDP and NBG (F=21.9243 , DF=2 ,

P ≤ 0.05). Samples from DDP had the most average number of leaves (16.60 ± 10.31),

while NBG had the least amount of average leaves (7.2 ± 4.15b), although this mean

difference was not significant (F=2.63 , DF=2 , P ≤ 0.05).

Table 3 Comparison of growth properties of orchids growing on oak trees within
different site locations.

Sites	Height on the Tree (cm)	Shoot Length (cm)	# of Leaves
FTBG	120.30 ± 8.14b	17.40 ± 2.75a	13.20 ± 2.39ab
DDP	217.40 ± 55.33a	7.50 ± 2.57b	16.60 ± 10.31a
NBG	94.00 ± 71.72b	7.92 ± 2.70b	7.2 ± 4.15b

Independent variable is characterized as the site location the orchid growing on oak was
sampled from (n=5 at FTBG, n=5 at DDP and n=5 at NBG). Values are expressed as
Mean ± Standard Deviation. One-way ANOVAs were performed on the mean value of
the factor evaluated. Means within a column followed by the same letter are not
significantly different at p ≤ 0.0500. Significantly different treatments were highlighted
by adding a letter next to the mean value.

The was significant differences across the plant health parameters at different site locations except for when it came to ascomycetes count. Basidiomycetes and ascomycetes count were based on the number of OTUs recovered per sample, for some root samples mycorrhiza was not recovered for many reasons although mycorrhiza may have been present. All 3 sites significantly influenced chlorophyll content in the leaves where FTBG had the greatest SPAD (65.02 ± 5.45a) and DDP had the least (30.90 ± 6.38c), all 3 sites were significantly different (F=26.6956, DF=2, P ≤ 0.05). FTBG NDVI values were also significantly higher than at DDP and NBG (F= 23.9122, DF=2, P ≤ 0.05).Basidiomycetes count was significantly higher at NBG when compared to FTBG and DDP (F= 6.6429, F=2, P ≤ 0.05).

Table 4 Comparison of plant health parameters of orchids growing on oak trees within different site locations.

Sites	SPAD values	NDVI values	Basidiomycetes	Ascomycete
FTBG	65.02 ± 5.45a	0.63 ± 0.07a	0.40 ± 0.55b	0.40 ± 0.55a
DDP	30.90 ± 6.38c	0.43 ± 0.02b	1.20 ± 0.45b	0.8 ± 1.10a
NBG	41.48 ± 10.05b	0.45 ± 0.04b	2.60 ± 1.52a	0.40 ± 0.89a

Independent variable is characterized as the site location the orchid growing on oak was sampled from (n=5 at FTBG, n=5 at DDP and n=5 at NBG). Values are expressed as Mean ± Standard Deviation. One-way ANOVAs were performed on the mean value of the factor evaluated. Means within a column followed by the same letter are not significantly different at p ≤ 0.0500. Significantly different treatments were highlighted by adding a letter next to the mean value.

4.3 Correlation Table

SPAD values did not show a correlation to the host tree the orchid was sampled from. NDVI values showed a positive correlation for palm trees and a negative correlation for cypress trees, and these were the only values with significance to the host trees. As the SPAD levels and NDVI values increased, the light levels measured decreased significantly and average leaf length showed an increase as well. Ascomycetes count was negatively correlated with NDVI values. Greater levels of height on the tree resulted in significantly less leaf and root average lengths. There was a positive correlation between average leaf length and root length. There was a negative correlation between average root length and basidiomycetes count.

Table 5 Bivariate correlation of all recorded orchid growth parameters.

	NDVI[a]	Light	Height on tree	Leaf Length	Root Length	No. of leaves	Basidio -mycetes	Asco -mycetes
SPAD	.660**	-.647*	-0.301	.687**	0.389	-0.058	0.032	-0.153
NDVI		-.730*	-0.281	.695**	0.428	0.262	-0.178	-.416*
Light Intensity			-0.304	-.777**	-0.997	-.687*	0.375	-0.016
Height on tree				-.440*	-.641**	0.21	-0.172	0.2
Leaf Length					.594*	-0.12	-0.232	-0.202
Root Length						0.114	-.531*	-0.145
No. of leaves							-0.235	0.131
Basidio -mycetes								-0.022

4.4 Mycorrhizal Diversity

There are 42 strains of mycorrhizal fungi that were obtained and analyzed, including 27 basidiomycetes (Table 6) and 15 ascomycetes OTUs (Table 7). These OTUs were obtained at the 97% sequence similarity threshold. Table 6 and 7 shows the occurrence of different OTUs among 3 different site locations as well as 3 distinct host tree species. 26 samples of mycorrhizal fungi were classified as *Tullasnella,* 3 were *Fusarium*, 2 were classified as *Anthrinium,* 8 as *Alternaria*, 2 as *Talaromyces,* 1 as *Neurospora* and 1 classified as *Sordaria. Tulasnella* was found in all 3 host species. Oak trees contained the ascomycetes *Fusarium, Anthrinium, Alternaria* and *Taloromyces*. Samples of *Sordaria, Alternaria and Neurospora* were found in orchid roots on cypress trees. Ascomycetes from palm trees were not recovered.

Table 6 List of fungal operational taxonomic units (OTUs) identified used to create Basidiomycetes phylogenetic trees.

ID	Location	Species	Host Tree	Primer	Outplant or natural	Related Taxanomic Group	Accession Number
R8	FTBG	ET	Oak	C1	N	*Tulasnella*	MH064468.1
F10	FTBG	ET	Oak	C2	N	*Ipomoea* Chloroplast	NC_042939.1
R5	FTBG	ET	Palm	C2	O	*Tulasnella*	MH064468.1

R4	FTBG	ET	Palm	C1	O	*Tulasnella*	MH064472.1
R3	FTBG	ET	Palm	B2	O	*Tulasnella*	MH064467.1
R2	FTBG	ET	Palm	C2	O	*Tulasnella*	MH064468.1
R2	FTBG	ET	Palm	B2	O	*Tulasnella*	MH064468.1
R1	FTBG	ET	Palm	C1	O	*Tulasnella*	MH064468.1
D9	DDP	ET	Cypress	C2	O	*Tulasnella*	MH064466.1
D8	DDP	ET	Cypress	C2	O	*Tulasnella*	MH064467.1
D7	DDP	ET	Cypress	C2	O	*Tulasnella*	MH064468.1
D6	DDP	ET	Cypress	C2	O	*Tulasnella*	MH064468.1
L4 5-2	DDP	ET	Oak	C2	O	*Tulasnella*	MH064468.1
L3 5-1	DDP	ET	Oak	C1	O	*Tulasnella*	MH064468.1
D5	DDP	ET	Oak	C1	O	*Tulasnella*	KM211339.1
D4	DDP	ET	Oak	C2	O	*Tulasnella*	KM211335.1
D3	DDP	ET	Oak	C1	O	*Tulasnella*	KM211339.1
D2	DDP	ET	Oak	C1	O	*Tulasnella*	KM211339.1
D1	DDP	ET	Oak	C1	O	*Tulasnella*	JF691303.1
N5B	NBG	ET	Oak	C2	N	*Tulasnella*	MH064466.1
N5B	NBG	ET	Oak	B2	N	*Tulasnella*	MH064468.1
N5	NBG	ET	Oak	C2	N	*Tulasnella*	MH064466.1
N5	NBG	ET	Oak	B2	N	*Tulasnella*	MH064466.1
N2	NBG	ET	Oak	C1	N	*Tulasnella*	MH064468.1

Source	Location	Species	Host Tree	Primer	Outplant or natural	Related Taxanomic Group	Genbank Accession Number
N2	NBG	ET	Oak	B2	N	*Tulasnella*	MH064468.1
N1	NBG	ET	Oak	C1	N	*Tulasnella*	MH064468.1
L6 4-2	NBG	ET	Oak	C2	N	*Tulasnella*	MH064466.1

Fungi were grouped into OTUs defined by 97% internal transcribed spacer (ITS) sequence similarity.

Table 7 List of fungal operational taxonomic units (OTUs) identified used to create Ascomycetes phylogenetic trees.

Source	Location	Species	Host Tree	Primer	Outplant or natural	Related Taxanomic Group	Genbank Accession Number
Root	FTBG	ET	Oak	A1	O	*Fusarium*	MN540868.1
F10	FTBG	ET	Oak	B2	N	*Anthrinium*	KF746108.1
F10	FTBG	ET	Oak	B1	N	*Arthrinium*	KF746108.1
L4 5-2	DDP	ET	Oak	A1	O	*Fusarium*	MT447548.1
L3 5-1	DDP	ET	Oak	A2	O	*Fusarium*	MT447548.1
D9	DDP	ET	Cypress	A2	O	*Sordaria*	MN341415.1
D9	DDP	ET	Cypress	B1	O	*Neurospora*	MH675494.1
D8	DDP	ET	Cypress	B2	O	*Alternaria*	MK649900.1
D8	DDP	ET	Cypress	A1	O	*Alternatia*	MT635195.1
D7	DDP	ET	Cypress	A1	O	*Alternaria*	MW478644.1
D7	DDP	ET	Cypress	B1	O	*Alternaria*	MW764299.1
D1	DDP	ET	Oak	B1	O	*Alternaria*	MT557073.1
D1	DDP	ET	Oak	A1	O	*Alternatia*	MN615420.1
L6 4-2	NBG	ET	Oak	A2	N	*Talaromyces*	MH856399.1
L5 4-2	NBG	ET	oak	A1	N	*Talaromyces*	MK849928.1

Fungi were grouped into OTUs defined by 97% internal transcribed spacer (ITS) sequence similarity.

Figure 6 Phylogenetic tree of Basidiomycetes sequences obtained from *Encyclia*
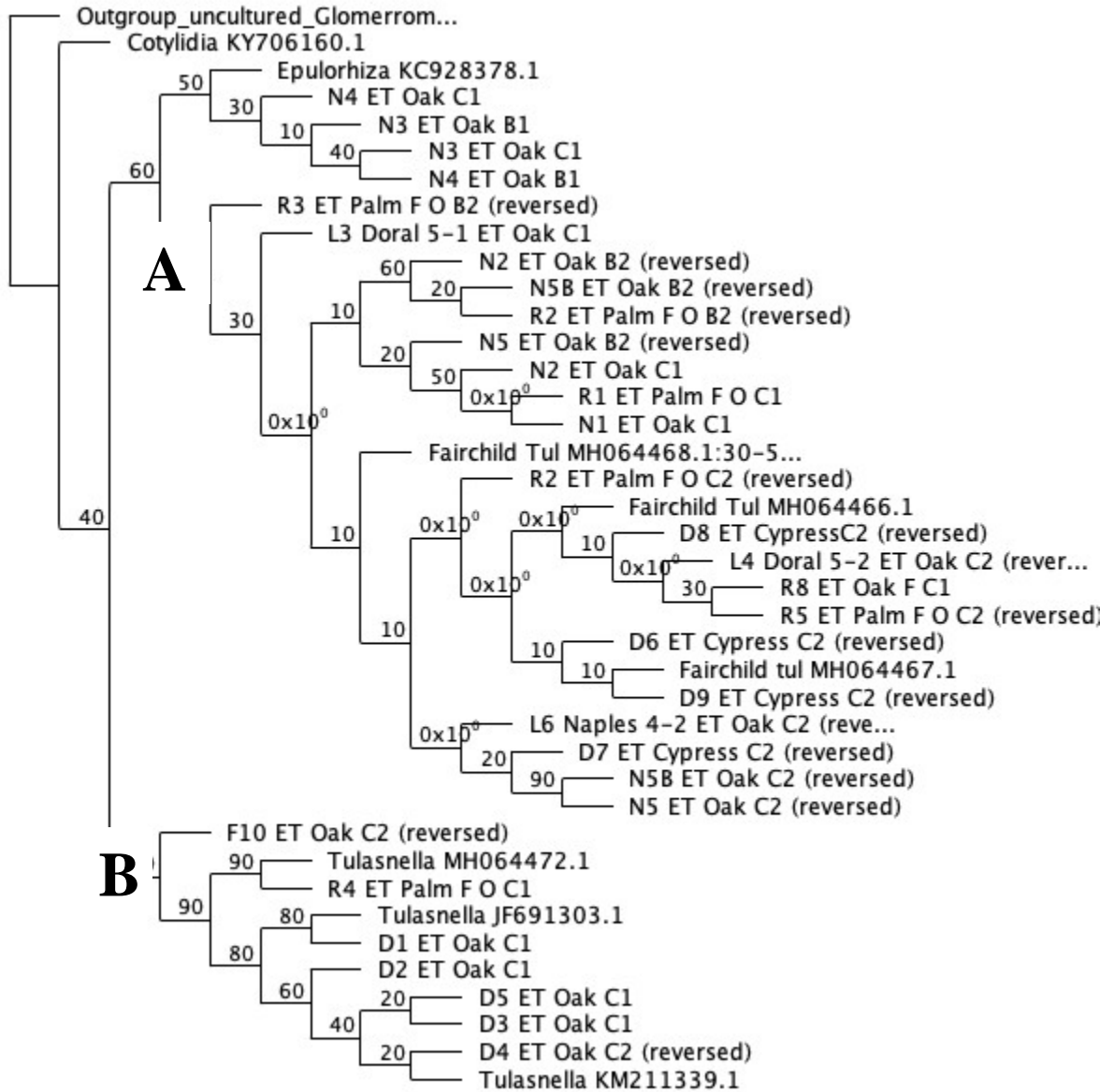
tampensis roots.

Maximum likelihood trees of ITS sequences of Basidiomycetes mycorrhizal fungi
isolated from *Encyclia tampensis* on 3 different host trees: oak, palm and cypress and
within 3 different site locations: FTBG, DDP, and NBG. Depicted are the relationships
among clades
A and B. Numbers show the bootstrapping percentage that supports the branches.

Figure 7 Phylogenetic tree of Ascomycetes sequences obtained from *Encyclia tampensis*
roots
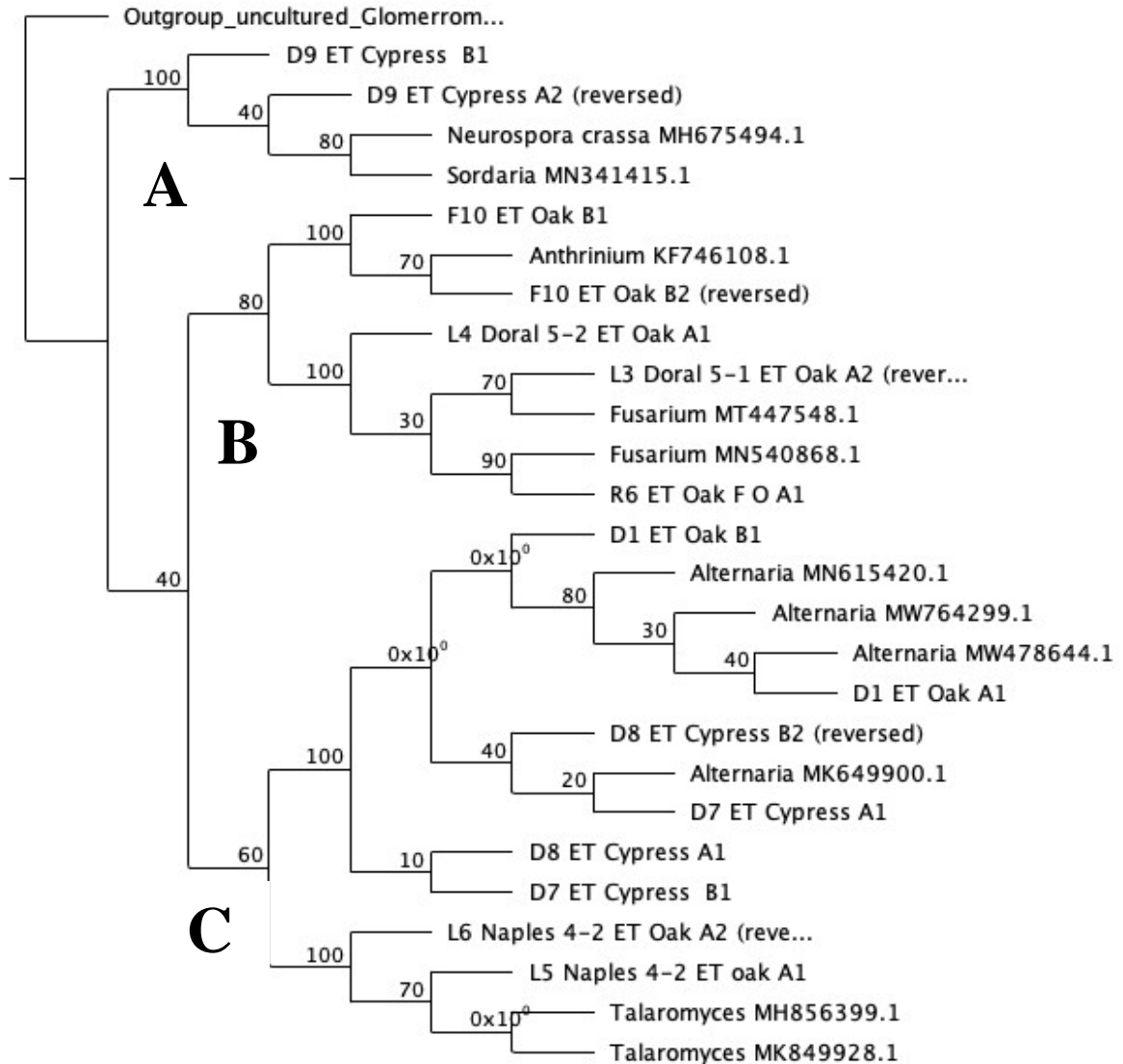
Maximum likelihood trees of ITS sequences of Basidiomycetes mycorrhizal fungi isolated from *Encyclia tampensis* on 3 different host trees: oak, palm and cypress and within 3 different site locations: FTBG, DDP, and NBG. Depicted are the relationships among clades
A, B, and C. Numbers show the bootstrapping percentage that supports the branches.

In Figure 6, Clade A contains samples collected from all 3 sites and across all 3

host tree species. Clade B contains samples from FTBG and DDP mainly from *Encyclia*

tampensis sampled from oak trees with the exception of one sample collected from orchid

root attached to a palm tree. Outplants and naturally occurring samples were found

between both clades (Figure 6). Figure 7 contains 3 clades where clade A contains

orchids sampled from Doral cypress trees. Clade B represents samples with high

relatedness to *Anthrinium* and *Fusarium* sampled from FTBG and DDP oak trees, both

naturally occurring and outplants. Clade B exhibits samples from DDP cypress and oak

trees and NBG oak trees belonging to *Alternaria* and *Talaromyces* taxonomic groups

(Figure 7). Samples belonging to the Ascomycetes phylum from palm trees were not

recovered.

5. DISCUSSION

The main purpose of this study was to determine if the site location and/or host tree type had an influence on *Encyclia tampensis* growth properties, plant health parameters, or ability to establish mycorrhizal associations and evaluate how this could be used to enhance reintroductions of orchids into their native ranges. The results indicate that palm trees are effective host trees in urban areas supported by the data showing those samples contained the greatest average leaf length as well as SPAD and NDVI values for orchids. The data suggests that orchid mycorrhizal fungi is widely available for uptake by *Encyclia tampensis* in south Florida urban environments.

For the analysis of the host tree's effects on biotic and abiotic factors, palm trees showed to be the most promising for planting due to high SPAD and NDVI values, coupled with low ascomycetes count which are potential pathogens that negatively affect plant growth and survivorship. When it came to plant health parameters however, chlorophyll content was significantly higher in palm trees than oak and cypress trees according to both the SPAD and NDVI values from the GreenSeeker™ device. It is a beneficial component to monitor the optical activity of chlorophyll molecules for quantification in plants as a way to estimate the effects of different stress factors both biotic and abiotic (Danijela et al., 2015).

FTBG oak trees had samples with the greatest average of SPAD values, NDVI values, and shoot length compared to DDP and NBG which could be due to the age and size of the tree. Previous studies have shown that microclimate conditions can alter chlorophyll fluorescence measures in tropical epiphytic measures when they were tested in conditions that were drier and hotter than natural conditions (Crain and Tremblay,

2017). A study on Green Fly Orchid host tree selection concluded that the most common host was Southern Magnolia with a much larger diameter and presumably older than the average available trees and is critical to the viability of the orchid population (Bergstrom and Carter, 2008). However, DDP was shown to have the greatest number of leaves even though it had the lowest SPAD values and the greatest average height on the tree. NBG had the greatest average number of OMF taxa, and there was no significant difference between means when it came to ascomycetes OTU counts between sites. When comparing data collected from *E. tampensis* at different site locations, those that were observed FTBG to be on large old growth oak trees were the most successful due to having high SPAD and NDVI values and the greatest shoot length. Results from DDP are indicative that samples with an average of 217.40 cm in height on the oak tree also had the greatest number of leaves.

The correlation table (Table 5) further confirms the ANOVA analysis where palm had the greatest NDVI values. NDVI values were also correlated with a greater leaf length and negatively correlated with ascomycetes association. A study done on nursery grown orchids used the NDVI values to plan for precision pesticide spraying against harmful pathogens where healthy orchids and infected orchids had significantly different values (Samseemoung et al., 2017). High light intensity was negatively correlated with leaf length and number of leaves, this is in agreement with a previous study where the optimal light intensity for Phalaenopsis was between 200 and 300 µmol·m–2·s–1 with photo inhibition and reduced carbon dioxide fication occurring above 400 µmol·m–2·s–1 (Lin et al., 2019). Moreover, root average was negatively correlated with basidiomycetes count.

To assess the effect of OMF, *in situ* root samples were collected to obtain fungal cultures and distinct fungal OTUs were identified using three fungi specific ITS primers that effectively screen for the Kingdom Fungi, orchid mycorrhizal fungi, and the genus *Tulasnella. E. tampensis* was seen to associate predominantly with two closely related clades of *Tulasnella*. The *Tulasnella* OTUs identified from outplants are consistent with those identified in the naturally occurring populations, indicating that urban/ non-natural areas are able to support the appropriate OMF across different native host trees; palm, oak and cypress species.

Knowing the presence and identity of OMF on varying host trees and at different sites within orchid roots can facilitate the process of selecting suitable habitats and compatible orchid species for translocation purposes. Some orchids exhibit differing levels of mycorrhizal specificity. Two distinct clades were found in the Tullasnellaceae family. Samples on oak, palm, and cypress across all 3 sites were found within one clade. The other clade only contained roots sampled from DDP on oak trees and one sample from FTBG on a palm tree. This shows the high mycorrhizal specificity of *Encyclia tampensis*. Knowledge on this specificity can influence a species' suitability for *ex situ* conservation. Ascomycetes were also recovered from root samples encompassing 3 distinct clades. These included *Nuerospora, Anthrinium, Fusarium, Alternaria* and *Talaromyces.* These are known as potential pathogens causing leaf spotting, root rot, and wilt (Samseemoung et al., 2017). Future studies are needed to understand the effect of root pathogens on plant health and how to best mitigate these effects with remedies such as pruning and chemical treatments.

The bivariate correlation analysis also shows that there is negative correlation between NDVI values and ascomycetes count. Ascomycetes fungi, considered putative root pathogens here, were significantly higher in hardwood trees than in palm trees. This shows a species ability to establish, and spread is also influenced by different biotic interactions in the range. A recent study found that native orchids were more likely to harbor pathogenic fungal groups, than co-occurring non-native congeners suggesting enemy release hypothesis also plays a role in orchid's naturalization and reintroductions (Downing et al., 2020). The enemy release theory states that invasive species are less impacted by enemies than native species because they are freed from parasites that keep their growth in check in their native environment (Middleton, 2019).

6. CONCLUSION

The host tree type had a significant effect on SPAD and NDVI values and presence of root pathogens but not OMF in palm trees, indicating it may be the best host tree for *E. tampensis* establishment and health in reintroduction programs. Outplants on oak trees in urban areas are comparable to those in naturally occurring habitats, and it is beneficial to protect large and old growth live oak trees that may support a greater abundance of orchids and support greater orchid growth in these spaces. Across all sites (urban park, botanical garden, and pine rockland natural habitat) and host trees (palm, oak and cypress), *E. tampensis* predominantly associated with *Tulasnella* mycorrhizal fungi, which indicates the suitability of urban spaces as well-suited habitats to support orchid germination and support growth. Conservation horticulture management practices in urban spaces are imperative to contribute to biodiversity preservation. The investigation of the obligate dependence of orchids on the association with mycorrhizal fungi is a critical component for the success of breeding programs and essential to orchid biology, ecology, and conservation.

Limitations of this study were found in sample sizes, research sites and number of orchid species samples. Recommendations for future studies are to increase these factors as well as to conduct lab germination tests and cryopreservation of OMF recovered and cultured in agar and liquid media obtained from orchid roots. This would allow for the understanding if OMF enabling germination is present in urban areas. Another possibility is seed baiting techniques where seeds are placed on bark to test germination as well as bark analysis for porosity, nutrients, and identifying the mycorrhizal community present. Cryopreservation would allow for OMF recovered to be stored long-term for future

experiments and for conservation purposes. Lab germination tests could be run to measure growth and to determine whether specific OMF are more effective at a quicker germination and enhanced seedling growth. Not only can OMF strains be used in germination tests but ascomycetes should also be tested for this. The effect of ascomycetes on orchid seedling growth would also be beneficial to identify how these pathogens affect orchid growth and the best suitable plan for mitigating any subsequent health effects. Testing samples from additional natural habitats such as in Everglades National Park and Big Cypress National Preserve would also prove to give insight into the function of orchids as a potential ecosystem health indicator species and how to better protect vulnerable species to enable endangered and threatened species to return to a functional, secure, and increasingly abundant population.

The findings of this study indicate the presence of OMF across multiple host trees and site locations and demonstrates these as potential suitable orchid habitats. Cevallos et al. (2017) noted the influence of keystone species and the way in which mycorrhizal communities and the way in which it is built around a core OMF species. This shows a fundamental role in mycorrhizal assembly and conservation is needed to prioritize keystone OMF that enable orchid germination and subsequent growth. Research is imperative for knowledge on the biotic and abiotic conditions affecting orchid growth and how this influences their geographical distribution and niche requirements. With appropriate planning, urban environments can serve as viable habitats for epiphytic orchid species and help mitigate the risk of biodiversity loss.

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