May 2010

Abundance of arbuscular mycorrhizae in epiphytic Orchidaceae : abiotic, biotic and taxonomic factors

Logan, E Smestad

Abundance of arbuscular mycorrhizae in epiphytic Orchidaceae: abiotic, biotic and taxonomic factors

Logan E Smestad

Department of Biology, Gustavus Adolphus College

ABSTRACT

Despite nutrient stress, not all epiphytes have mycorrhizal associations, including some epiphytic orchids (Lesica & Antibus 1990). In this study epiphytic orchids were collected from tree and limb falls in the Monteverde Cloud Forest, Costa Rica. Abiotic factors such as branch circumference, presence or absence of moss mats, thickness of moss mats, substrate pH and moisture content were measured for their impact on mycorrhizal abundance. Biotic factors such as relative age of the orchid, plant height and the presence or absence of pseudobulbs were also considered. Finally taxonomic factors were assessed as abundance of mycorrhizae related to the Pleurothallid or non-Pleurothallid subtribes. Unlike Lesica and Antibus (1990) who found less than half of the collected Orchidaceae species with mycorrhizal associations, I found them to be nearly ubiquitous. Of the 50 samples taken from a variety of orchid species, 46 of them showed mycorrhizal associations. Abiotic factors, taxonomic factors, age of the orchid, and plant height had no obvious bearing mycorrhizal abundance. However, orchids with pseudobulbs had fewer mycorrhizal hyphae. Therefore orchids lacking mycorrhizae may compensate with features like pseudobulbs, which store nutrients, but their near ubiquity suggests that most orchids rely on mycorrhizal fungi to increase nutrient uptake.

RESUMEN

A pesar del estrés nutricional, no todas las epífitas tienen asociaciones de micorrizas, entre ellas algunas orquídeas epífitas (Lesica & Antibus 1990). En este estudio se colectaron orquídeas epífitas de los árboles y las extremidades caídas en el Bosque Nuboso de Monteverde, Costa Rica. Factores abióticos, como la circunferencia de la rama, la presencia o ausencia de los tapetes de musgo, el espesor de los tapetes de musgo, el substrato de pH y contenido de humedad se realizaron mediciones de su impacto sobre la abundancia de micorrizas. Los factores bióticos como la edad relativa de la altura de planta de la orquídea, y la presencia o ausencia de pseudobulbos también fueron considerados. Finalmente se evaluaron los factores taxonómica como la abundancia de micorrizas en relación con orquídeas Pleurothalidas o non-Pleurothallid subtribus. A diferencia de Lesica y Antibus (1990) que encontró menos de la mitad de las especies de Orchidaceae con las asociaciones de micorrizas, encontré en este estudio que son casi omnipresentes. De las 50 muestras tomadas de una variedad de especies de orquídeas, 46 de ellos mostraron asociaciones que forman micorrizas. Factores abióticos, factores taxonómica, la edad de la orquídea, y altura de planta no obvia la abundancia de micorrizas. Sin embargo, las orquídeas con pseudobulbos tenía menos hifas del hongo. Por eso las orquídeas que carecen de micorrizas pueden compensar con características como pseudobulbos, que almacenan nutrientes, pero su casi ubicuidad sugiere que la mayoría de las orquídeas se basan en hongos micorrícicos para aumentar la absorción de nutrientes.

INTRODUCTION

It is well known that mycorrhizal associations are mutualistic relationships between plants and fungi; and over 92% of vascular plants require these associations for much of their nutrient

uptake (Huynh *et al.* 2009, Lesica & Antibus 1990, McCormick *et al.* 2006). In this symbiosis, mycorrhizae receive photosynthetic products and domatium within its host's roots, while plants receive an enhanced uptake of water and organic nutrients (Harley & Smith 1983). One specific type of mycorrhizae associated with vascular trees and epiphytes is that of arbuscular mycorrhizae (AM); it is ever-present in both temperate and tropical habitats and is generally associated with nutrient poor environments (Gerdemann 1968, Janos 1980, Lesica & Antibus 1986, Lesica & Antibus 1990).

There are an estimated 20,000 to 35,000 species in the Orchidaceae family (Cribb *et al*. 2003), and of these most of their success can be attributed these symbiotic associations with mycorrhizal fungi (Leake 1994). Nearly all orchid species require these associations during germination. Orchid seeds have no endosperm and pass through an initial non-photosynthetic, mycoheterotropic/mycotrophic seed phase. During this phase orchids get all of their organic carbon from mycorrhizae through symbiotic exchange or by digesting intracellular arbuscular mycorrhizal (AM) fungal pelotons (coils of fungal hyphae) (Alexander & Hadley 1985, Rasmussen 2002). A vast majority of these orchid species continue to maintain mycorrhizal associations later in life, even after they have grown photosynthetic leaves. Post germination, AM fungal associations continue to supply orchids with nutrients other than carbon, including nitrogen and phosphorus (Cameron *et al.* 2006, Cameron et al. 2007, Smith 1966).

Lesica and Antibus (1990) studied a variety of vascular epiphytes for the incidence of mycorrhizae in La Selva and Monteverde Costa Rica. They found a few species of orchids (*Elleanthus graminifolius, Epidendrum octomerioides, Maxillaria hedwigae, Maxillaria uncata, Platystele microtatantha, Scaphyglottis prolifera, Stelis aprica and Stelis endresii*) that did not have mycorrhizal associations. Unfortunately, these authors did not list the numbers of samples they collected from these species. Yet, they surmised that these orchids lacked mycorrhizae because of certain ecologic or taxonomic factors that may have caused failure in the development of AM fungi. Abiotic factors, such as dryness and exposure in certain epiphytic habitats, may also have played a role in their failure to recruit or maintain these fungal associations. Moreover, species of orchids that live on the exposed and bare limb habitats have low rates of photosynthesis (Benzing 1986). Therefore the species of orchids that were found without mycorrhizal associations may have had insufficient amounts of resources to support the mycorrhizal symbiosis (Lesica & Antibus 1990).

In this study, a variety of genera from the family Orchidaceae were examined for the presence of AM hyphal associations based on both abiotic and taxonomic factors that were suggested within the study done by Lesica and Antibus. Substrate type, branch circumference, canopy mat thickness, individual plant height and plant taxonomy were determined and compared to the relative percent area coverage of mycorrhizae contained within each orchid.

METHODS

Study Sites

Orchid roots were collected from the La Estación Biologica Monteverde Reserve near Monteverde, Puntarenas, Costa Rica (10°18'N, 84°49'E). The forest is characterized as a Premontane Wet Forest using Holdridge life zones and receives an average of 2.0 - 2.5 meters of rainfall each year (Haber *et al.* 2000). The Monteverde area also receives an additional half-meter or more of mist each year, further characterizing the area surrounding the reserve as a

Cloud Forest. On the Pacific slope of the Cordillera de Tilarán, orchid individuals were collected at an elevation of 1550 meters. Samples were selected from fallen branches and trees within the forest at various locations in the station reserve. Specimens were collected from 15 April 2010 to 20 April 2010, which is the end of the four to five month dry-season.

Field Methods

A total of 50 orchid individuals were sampled from within the Biological Station Reserve from numerous tree and limb falls. Of these specimens, only those that had not previously touched the ground and did not show any physical damage were collected; in this way it was assured that an accurate depiction of these organisms was represented. From each orchid, a sample measuring roughly three centimeters was traced back to the parent plant, cut and placed in a vial containing ethanol in order to preserve the specimen for later analysis. For each sample, I defined the substrate mat as either 'bare branch' or 'moss mat'. In bare branch habitats the orchid roots were wrapped around the limb or trunk, while in moss mat substrate habitats the orchids grew out of a dense moss mat. The thickness of these mats was also quantified using a standard caliper. In addition, I measured plant height for each orchid sample from the base of the plant to the top of the last leaf or inflorescence, with a comparison of height based on species type made at a later time. A measurement of the circumference for each branch was also taken by measuring directly below the orchid that each root sample was taken from. I determined soil pH and moisture content using a Rapitest® 4-Way Analyzer. I also took photos of each orchid for identification. Evidence of previously flowering, senescence of the pseudobulbs and size of the individual was used to determine the relative age of the orchid.

Laboratory Methods

Protocols for clearing and staining AM fungi were adapted from several sources (Bagyaraj & Stürmer 2008, Lee 2006, Phillips & Hayman 1970, Weller 2002). Roots collected from each orchid specimen were removed from their ethanol storage solution and then washed thoroughly with water to remove any soil or debris. They were then placed in a 2% KOH bath at 90°C in an incubation oven for 90 minutes. Once they had sufficiently cleared, the roots were washed with water to remove any excess KOH. If any of the samples were still too darkly pigmented, I soaked them in an alkaline H_2O_2 solution (3 mL 20% NH_4OH, 30 mL 3% H_2O_2, 567 mL tap water) for up to 20 minutes or until clear. After soaking the roots in the alkaline H_2O_2 solution, the roots were again rinsed thoroughly with water. The cleared root samples were then placed into a 1% HCl solution for five minutes at room temperature to acidify them for proper staining. After this the samples were immediately placed into a 4:2:1 acidic glycerol solution containing trypan blue (50% glycerol : 1% HCl : 0.05% trypan blue) at 90°C for one hour. Once staining was complete, I placed the root samples in a solution of 50% glycerol : 1% HCl for up to 24 hours before viewing with a compound microscope.

Slides of each sample were prepared by making thin 0.2 – 0.5 millimeter transverse cross-sections of each root. Clear nail polish was used to paint around the cover slip of each slide to prevent desiccation of the root sample. Once all of the slides were processed, they were photographed under a compound microscope at 400X magnification using an Olympus 7.1 mega pixel digital camera with a standardized zoom setting and an attached microscope adaptor. I then analyzed each of these photographs for percent area coverage of hyphae and arbuscles using the

program ImageJ64. Within this program, each of the hyphal or arbuscular structures was painted over and then converted to a binary image. ImageJ64 was then able to calculate the percent area of the total coverage of mycorrhizal structures within each of the photos. Thus, the percent area of mycorrhizal infection was able to be determined for each plant.

RESULTS

From orchid specimens collected, 31 different species were identified (Appendix i). None of these samples were of the same species that Lesica and Antibus (1990) used during their study, however, it is possible that some of the unidentified individuals could have been of the same species. Additionally some of the orchid species that were collected in my study were of the same subtribe as few of the species found by Lesica and Antibus (1990). Of the 50 individuals collected very few (N = 4) did not have mycorrhizal infections. Of these four uninfected individuals, three were older orchids while one was classified as a juvenile. The older orchids were found growing on a bare branch environment whereas the non-adult individual was growing on a mossy substrate mat.

Influence of abiotic factors on mycorrhizal infections

AM associations were found in 46 of the 50 total collected Orchidaceae samples. The overall abundance of the mycorrhizae found infecting these orchids was not shown to be related to the circumference of the fallen branch or tree on which they were growing ($R^2 = 0.071424$, $p = 0.0606$) (Fig. 1).

The abundance of these fungi infecting orchids was not strongly ($p = 0.4484$) affected by the presence (MEAN 3.17011 ± 2.0859 SD, N = 18) or absence (MEAN 3.7609 ± 2.92062 SD, N = 32) of moss mats (Fig. 2). Even in the presence of moss mats, the comparative thickness of these mats was not associated with the level of infection of the AM fungi found within the roots of the orchids ($R^2 = 0.004181$, $p = 0.2015$) (Fig. 3). There was also a high variation of mycorrhizal abundances found within those specimens growing on the bare substrate mat with a thickness of zero (Fig. 3).

Both pH and moisture content were consistent throughout all orchid specimens collected (pH = 7, moisture content = 1.5). Thus, there was little correlation between these values when compared to the percent area of mycorrhizal infections found within each specimen.

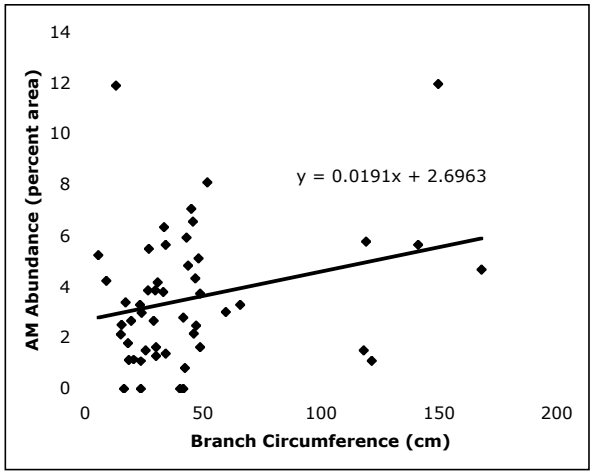

Figure 1. Abundance of mycorrhizal measured as percent area under 400x magnification compared to the branch circumference. Samples were collected in Monteverde, Costa Rica, 15 April 2010 to 20 April 2010. N = 50. Correlations between hyphal infections and branch circumference were not found to be statistically significant ($R^2 = 0.071424$, p = 0.0606).

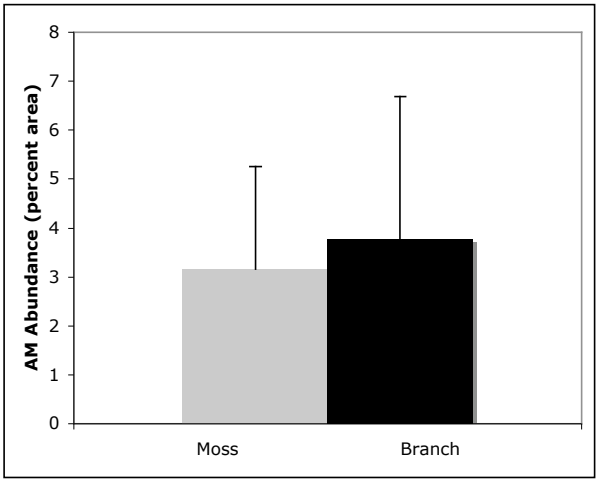

Figure 2. Abundance of the mycorrhizal hyphae measured as percent area under 400x magnification compared to the presence or absence of a moss mat (moss: N = 18, branch: N = 32). Samples were collected in Monteverde, Costa Rica, 15 April 2010 to 20 April 2010. Hyphal infections between these two habitats were not found to be significantly different (F ratio = 0.5843, p = 0.4484). Bars represent means + standard deviations.

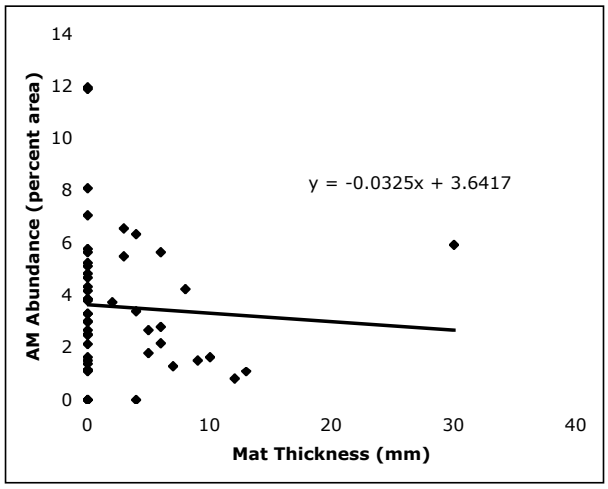

Figure 3. Abundance of mycorrhizal hyphae measured as percent area under 400x magnification compared to the thickness of the moss mat (mm). Samples were collected in Monteverde, Costa Rica 15 April 2010 to 20 April 2010. N = 50. Correlations between hyphal infections and the thickness of the orchid moss mats were not found to be significant ($R^2 = 0.004181$, p = 0.2015).

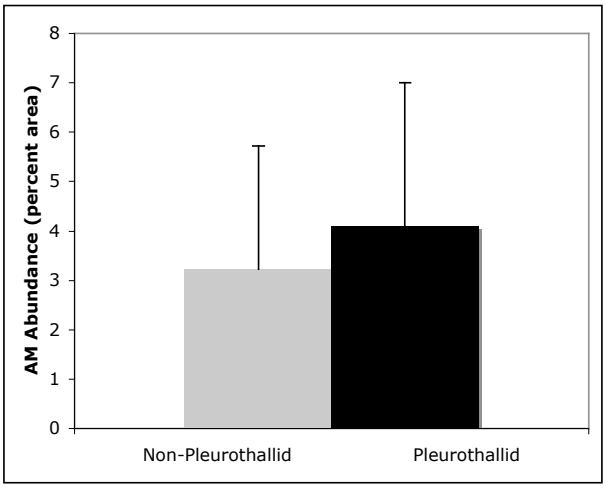

Figure 4. Abundance of mycorrhizal hyphae as measured as percent area under 400x magnification compared to the major subtribe (Pleurothallid: N = 13). Samples were collected in Monteverde, Costa Rica, 15 April 2010 to 20 April 2010. N = 50. The difference between the hyphal infections and this major subtribe were not found to be significant (F ratio = 1.2815, p = 0.2633). Bars represent means + standard deviations.

Influence of taxonomic factors on mycorrhizal infections

The differences in the two taxonomic groups (Pleurothallid MEAN 4.11556 ± 2.89048 SD, N = 13, non-Pleurothallid MEAN 3.23628 ± 2.48594 SD, N = 37) represented within the data were not associated with ($p = 0.2633$) the growth and abundance of the fungal hyphae found within the roots of the orchids (Fig. 4). Further analysis showed that there was equality in the variances between these two subtribes of Pleurothallid and non-Pleurothallid and their level of mycorrhizal infections. This suggests that these groups are within the same normal distribution (Bartlett's Test, F ratio = 0.5005, $p = 0.4793$).

Influence of biotic factors on mycorrhizal infections

In terms of the comparative age, (adult MEAN 3.7812 ± 2.70927 SD, N = 44, non-adult MEAN 1.878 ± 1.24626, N = 6) there was no evidence to suggest ($p = 0.0985$) that age influences the growth and presence of AM fungi (Fig. 5).

Although plant height was taken for each of the orchid specimens collected, these values were highly variable depending on the species of the orchid. Consequently there was little if any correlation of these values with those of the percent area infection of AM fungi found within the orchid roots. Nevertheless a relationship was found with the presence and absence of pseudobulbs. My data show that there were lower abundances of AM fungi in individuals that had pseudobulbs (MEAN 4.62209 ± 3.18939 SD, N = 22) in comparison to the specimens that did not have pseudobulbs (MEAN 2.71268 ± 1.76365 SD, N = 28) (Fig. 6).

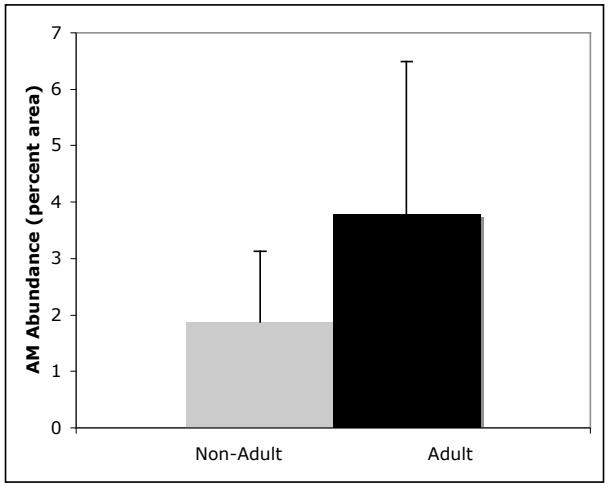

Figure 5. Abundance of mycorrhizal hyphae as measured as percent area under 400x magnification compared to the apparent age of the orchid. Samples were collected in Monteverde, Costa Rica 15 April 2010 to 20 April 2010. The difference in hyphal infections of the AM fungi between these two age categorizations (Non-Adult: N = 6 and Adult: N = 44) was not found to be significantly different (F ratio = 2.8387, $p = 0.0985$). Bars represent means + standard deviations.

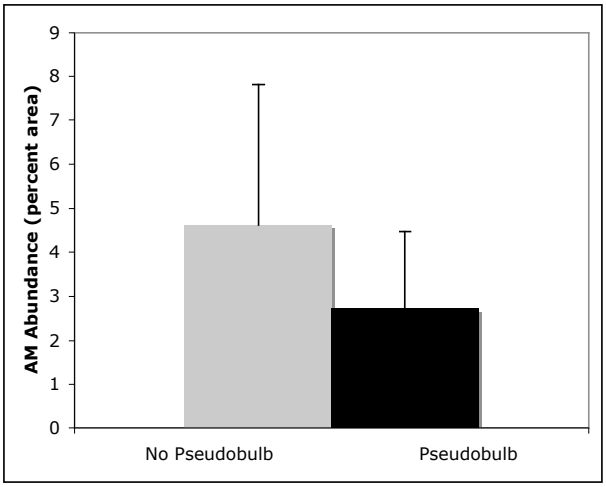

Figure 6. Abundance of mycorrhizal hyphae as measured as percent area under 400x magnification compared to the presence (N = 28) or absence (N = 22) of a pseudobulb. Samples were collected in Monteverde, Costa Rica, 15 April 2010 to 20 April 2010. The difference between AM hyphal densities and the presence or absence of a pseudobulb was found to be statistically significant (F ratio = 7.3447, $p = 0.0098$). Bars represent means + standard deviations.

DISCUSSION

In this study I found that there was little evidence to support the theories of Lesica and Antibus (1990) in their findings of orchids without mycorrhizal associations. Originally they had hypothesized that certain habitat environments as well as taxonomic factors of the host orchid may have caused failure in the development of AM fungi. Specifically, they speculated that orchids growing on bare and dry substrates would not be able to support the growth of the fungus and that individuals growing on bare limbs would have lower rates of photosynthesis (Benzing 1986) and thus be unable to support a symbiosis.

In this study I found that there was little influence of substrate mat composition or thickness, branch circumference, taxonomic differences of the orchid species or relative age of the orchid individual on the recruitment of AM fungal infections within roots of the Orchidaceae family. Only four individuals out of a total 50 were found without mycorrhizal infections. This strongly suggests and further substantiates the evidence of Leake (1994) that mycorrhizae are a requirement of each orchid individual. Data also suggest that adult and non-adult orchids do not differ in their mycorrhizal associations and they are needed regardless of orchid age or life stage.

Nonetheless, in accounting for the individuals that were not found with mycorrhizal associations, previous studies dealing with the effects of fungicides on the growth rate and productivity of orchids showed that without AM fungi, the orchids will grow, but at a reduced rate (Alexander & Hadley 1984). These treated orchids also had reduced levels of organic phosphate within their cells suggesting that mycorrhizae are able to help phosphorylate these inorganic phosphate molecules into organic phosphate in which the orchids can use (Simonis & Urbach 1973). Therefore it might be possible to state that orchids do not need AM fungal associations, at least post-germination/early development. As a result, orchids begin life with these associations, yet some are able to lose them once they are able to photosynthesize. If the orchids are old enough and able to produce enough of their own nutrients, it may survive without the symbiotic association of AM fungi. Though my results do show that this loss is unrelated to substrate composition or taxonomic factors.

It may also be possible to conclude that orchid individuals that have pseudobulbs are better able to store and access nutrients and are therefore less reliant on these AM fungal infections. Pseudobulbs are vital in the survival and reproduction of orchids as they are used in the storage of carbohydrates (Yong & Hew 1995). Studies with a species of *Oncidium* orchid showed that its pseudobulb is a strong sink for products of photosynthesis. In particular, this orchid relies on these sinks at certain stages of their life to promote reproduction and growth (Wang *et al.* 2008). These fungal infections, though beneficial to the orchid, also take photosynthates from the orchid. As previously stated, AM fungi do supply the orchid with carbon in the form of digested hyphae, consequently those orchids that have pseudobulbs may be better able to store carbohydrates; as a result they may rely less upon the mutualism with its mycorrhizae.

Another explanation may be that the four individuals without mycorrhizal associations were undergoing some radical change in their life cycles. Previous studies show the ability for orchids to change their symbiotic mycorrhizae species during times of extreme environmental stress (e.g. drought) during which the initial species of mycorrhizae died (McCormick *et al*. 2006). Since all of the samples I collected were from fallen branches or trees, some of these orchids may have been under a great deal of stress and thus in the process of changing their mycorrhizal symbionts. Since sample collection was performed at the end of a four to five

month dry season, this might have incurred even more stress upon these especially vulnerable individuals. In particular, the one non-adult individual might have been under the most strain due to it being a young orchid trying to establish itself when its host tree fell to the ground. This individual was also the only orchid without mycorrhizal associations found growing on a moss mat; the other three samples were found growing on a bare branch substrate.

Factors such as random chance and seasonality among a multitude of others could also have played a role in explaining why no mycorrhizae were found in four of my samples and in over half of the samples found by Lesica and Antibus (1990). Mycorrhizal fungi, their growth and their associations with their hosts are not widely understood, hence there may be other factors not accounted for (Hadley 1970). In order to study the relationship between these mycorrhizal symbionts and their host species in greater depth it is important to examine the exact situations in which orchids are forced to change their AM associations. It has been suggested that over its lifetime a single individual may be able to associate with more than one mycorrhizal species at once (polygamy) or may switch from one species to the next in a more serial manner (McCormick *et al*. 2006). Either way, it is important to establish greater insight as to how these individuals are able to switch their associations, especially among certain species. My data show that neither taxonomic status, substrate type, nor environmental habitat provide sufficient evidence to classify why some orchids are without mycorrhizal associations. As such, it appears that orchids without mycorrhizae are fairly rare. This demonstrates that further research is needed on other factors such as species interaction and specificity as well as the influence of certain environmental stressors in order to confirm the regulating processes for AM fungal associations in orchids.

ACKNOWLEDGEMENTS

I would like to thank my advisor Alan Masters for his time and patience in establishing a viable project for me. Also I would like to especially thank Karen Masters for her invaluable help in identifying each of my orchid samples. Thanks to the University of Georgia Ecolodge Station for letting me use their lab space over the week long time period it took me to stain all of my root samples.

LITERATURE CITED

ALEXANDER, C. AND G. HADLEY. 1984. The effect of mycorrhizal infection of *Goodyera repens* and its control by fungicide. New Phytologist 97: 391-400

ALEXANDER, C. AND G. HADLEY. 1985. Carbon movement between host and mycorrhizal endophyte during the development of the orchid *Goodyera repens*. New Phytologist 101: 657-665

BAGYARAJ, J. D. AND S. L. STÜRMER. Arbuscular Mycorrhizal Fungi (AMF). 2008. *In* F. M. S. MOREIRA, E. J. HUISING, AND D. E. BIGNELL, editors. A Handbook of Tropical Soil Biology: Sampling & Characterization of Below-ground Biodiversity, pp. 131-141. Earthscan, London, UK.

BENZING, D. H. 1986. The vegetative basis of vascularepiphytism. Selbyana 9: 23-43.

CAMERON, D. D., J. R. LEAKE, AND D. J. READ. 2006. Mutualistic mycorrhiza in orchids: evidence from plant – fungus carbon and nitrogen transfers in the green – leaved terrestrial orchid *Goodyera repens*. New Phytologist 171: 405-416.

CAMERON, D. D., I. JOHNSON, J. R. LEAKE, AND D. J. READ. 2007. Mycorrhizal Acquisition of Inorganic Phosphorus by the Green-leaved Terrestrial Orchid *Goodyera reopens*. Annals of Botany 99: 831-834.

CRIBB, P. J., S. P. LELL, K. W. DIXON, AND R. L. BARRETT. 2003. Orchid conservation: a global perspective. In: DIXON K. W., S. P. KELL, R. L. BARRETT, P. J. CRIBB eds. Orchid conservation. Natural History Publications (Borneo): Kota Kinabala, Sabah.

GERDEMANN, J. W. 1968. Vesicular-arbuscularmycorrhizaand plant growth. Annu. Rev. Phytopathol. 6: 397-418.

HABER, W. A., W. ZUCHOWSKI, AND E. BELLO. 2000. An Introduction to Cloud Forest Trees Monteverde, Costa Rica, 2nd edition. Mountain Gem Publications, Monteverde de Puntarenas, Costa Rica.

HADLEY, G. 1970. Non-specificity of symbiotic infection in orchid mycorrhiza. New Phytologist 69: 1015-1023.

HUYNH, T. T., R. THOMSON, C. B. MCLEAN, AND A. C. LAWRIE. 2009. Functional and genetic diversity of mycorrhizal fungi from single plants of Caladenia formosa (Orchidaceae). Annals of Botany 104: 757-765

HARLEY, J. L. AND S. E. SMITH. 1983. Mycorrhizal symbiosis. Academic Press, London.

HEW, C. S. AND N. G. CKY. 1996. Changes in mineral and carbohydrate content in pseudobulbs of C_3 epiphytic orchid hybrid *Oncidium* Goldiana at different growth stages. Lindleyana 11: 125-134.

JANOS, D. P. 1980. Mycorrhizaeinfluence tropical succession. Biotropica 12(Supplement): 56-64.

LEAKE, J. R. 1994. The biology of myco-heterotrophic (saprophytic) plants. New Phytologist 127: 171-216.

LEE, Y. Y. C. 2006. Mycorrhizae concentration and fitness of canopy orchids: *Masdevallia chasei* and *Trichosalpinx memer* in the Cloud Forest of Monteverde, Costa Rica. CIEE Tropical Ecology and Conservation Fall Proceedings.

LESICA, P. AND R. K. ANTIBUS. 1986 Mycorrhizaeof alpine fell-field communities on soils derived from crystalline and calcareousparent materials. Can. J. Bot. 64: 1691-1697.

LESICA, P. AND R. K. ANTIBUS. 1990. The Occurance of Mycorrhizae in Vascular Epiphytes of Two Costa Rican Rain Forests. Biotropica 22(3): 250-258.

MCCORMICK, M. K., D. F. WHIGHAM, D. SLOAN, K. O'MALLEY, B. HODKINSON. 2006. Orchid – fungus fidelity: a marriage meant to last? Ecology 87(4): 903-911.

PHILLIPS, J. M. AND D. S. HAYMAN. 1970. Improved procedures for cleaning roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi. Transactions of the British Mycological Society 55: 158-160.

RASMUSSEN, H. N. 1995. Terrestrial orchids from seed to mycotrophic plant. Cambridge University Press, Cambridge, UK.

SIMONIS, W. AND W. URBACH. 1973. Phosphorylation *in vivo*. Annual Review of Plant Physiology 24: 89-114.

SMITH, S. E. 1966. Physiology and ecology of orchid mycorrhizal fungi with reference to seedling nutrition. New Phytologist 65: 488-499

WANG, C. Y., C. Y. CHIOU, H. L. WANG, R. KRISHNAMURTHY, S. VENKATAGIRI, J. TAN, K. W. YEH. 2008. Carbohydrate mobilization and gene regulatory profile in the pseudobulb of *Oncidium* orchid during the flowering process. Planta 227:1063-1077.

WELLER, S. Y. 2002. Abundance of vesicular-arbuscular mycorrhiza in adult epiphytic orchidaceae between two canopy substrates. EAP Spring Symposium Proceedings.

Appendix i.

Species	Subtribe	Pseduobulb	Number	Mean of AM % Area	Std Dev
Brassia arcuigera	-	Y	1	7.062	0
Epidendrum firmum	-	N	1	11.909	0
Epidendrum species A	-	N	2	5.685	3.39411
Lepanthes ciliisepala	Pleurothallid	N	1	5.661	0
Masdevallia species A	Pleurothallid	N	1	2.47	0
Masdevallia striatella	Pleurothallid	N	3	7.6123	3.77376
Maxillaria bracteata	-	Y	1	0	0
Maxilaria inaudita	-	Y	10	2.0836	1.06143
Maxillaria species A	-	Y	1	5.227	0
Maxillaria species B	-	Y	1	4.68	0
Maxillaria species C	-	Y	1	1.391	0
Maxillaria species D	-	Y	1	3.013	0
Maxillaria species E	-	Y	1	5.486	0
Pleurothallis aristata	Pleurothallid	N	1	1.497	0
Pleurothallis cogniauxiana	Pleurothallid	N	2	2.411	3.40967
Pleurothallis dolichpus	Pleurothallid	N	1	5.917	0
Pleurothallis ruscifolia	Pleurothallid	N	1	6.347	0
Pleurothallis species A	Pleurothallid	N	2	1.3515	0.38113
Pleurothallis species B	Pleurothallid	N	1	2.516	0
Pleurothallis tuerckheimii	Pleurothallid	N	1	5.636	0
Prosthechea (Encyclia) campylostalix	-	Y	4	2.8438	1.92765
Prosthechea species A	-	Y	2	2.9105	1.7953
Prosthechea species B	-	Y	1	2.801	0
Prosthechea species C	-	Y	1	3.8	0
Restrepia species A	Pleurothallid	N	1	2.163	0
Scaphyglottis species A	-	Y	1	1.807	0
Species A	-	N	1	4.327	0
Speceis B	-	Y	1	2.656	0
Species C	-	Y	1	0	0
Stelis microchila	Pleurothallid	N	1	1.093	0
Stelis parvula	Pleurothallid	N	2	5.209	1.90212