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Transfer of Fungal Endophytes from Leaves to Woody Substrates

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ABSTRACT:

Fungal endophytes have been found in all plants surveyed to date, yet for many fungi the function of endophytism is still unknown. The Foraging Ascomycete Hypothesis (FAH) proposes that saprotrophic fungi utilize an endophytic stage in leaves to modify dispersal. Under this hypothesis, leaves can provide food and water during time of environmental scarcity and they can transport the fungi to other substrates upon dehiscence. If the FAH is accurate, then some endophytes should have the ability to colonize saprobic substrates directly from a leaf-endophyte stage, though this has been little studied. To assess this ability, twelve surface-sterilized leaves of a tropical tree (*Nectandra lineatifolia* Mez) were placed directly on wood and incubated for six weeks. Fungi from the wood were subsequently cultured and identified by ITS sequences or morphology. 477 fungal isolates comprising 26 OTUs were cultured from the wood, the majority of which belong to saprotrophic genera (70.8% of OTUs, 82.3% of isolates). The mean OTU richness per leaf was 5.67. The term *viaphyte* (literally, “by way of plant”) is introduced and defined as fungi that colonize living leaves as endophytes and use the leaves to transfer to another substrate, such as wood, when the leaves dehisce. These results strengthen the Foraging Ascomycete Hypothesis and expose the possibility that viaphytism plays a significant role in the dispersal of fungal saprotrophs.

Keywords: fungal endophytes; fungal dispersal; fungal culturing

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Introduction

Endophytes are defined as symptomless endosymbionts of living plant tissues (Stone 2000). Fungal endophytes are ubiquitously present in terrestrial plant tissues worldwide (Arnold and Lutzoni 2007). Every plant surveyed to date has turned up several to hundreds of species of fungal endophytes per individual, and a single plant species may host thousands of these symbionts across its entire range (Arnold and Lutzoni 2007, Rodriguez et al. 2009).

Fungal endophytes can have a range of consequences for their plant hosts' fitness, ranging from positive (mutualistic) to negative (pathogenic) (Rodriguez et al. 2009). While the effects of endophytes on hosts have garnered considerable attention in endophyte research, this paper is concerned with the opposite question: what is it about endophytism that benefits the fungi?

This question has garnered a variety of hypotheses although few studies have directly addressed them. One popular hypothesis is that endophytes are acting as "latent saprotrophs" and they benefit from being the first to colonize plant tissues after senescence or death of plant tissues (Promputtha et al. 2007, Parfitt et al. 2010, Porras-Alfaro and Bayman 2011, Szink et al. 2016). This partially explains why many endophytic taxa are known saprotrophs and is supported by the finding that many endophytes are capable of producing wood-decay enzymes (Urairuj et al. 2003, Oses et al. 2006, Promputtha et al. 2010). Some endophytes also benefit from direct vertical transmission to their host's offspring, which is common for clavicipitaceous (*i.e.*, grass) endophytes (Clay 1988, Hodgson et al. 2014). Finally, others may be latent pathogens waiting to exploit a weakened state of their host (Carroll 1988, Slippers and Wingfield 2007).

Yet, the benefits of endophytism remain unclear for most endophytes. For instance, a number of major wood decomposers have been found as endophytes in leaves (Promputtha et al.

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2007), but the benefits of leaf colonization for these taxa are not clear. The simplest explanation is that they spread there haphazardly through growth from adjacent woody tissues. However, it has been shown that endophyte communities are often different between twigs and leaves of the same host (Sun et al. 2011, Tateno et al. 2015), and the two tissues are likely colonized independently and through different mechanisms (Peršoh 2013). Foliar endophytism has thus been described as a "dead end" for these fungi (Bayman et al. 1998).

While the dead-end hypothesis proposes that foliar endophytism is simply an incidental fate for some fungi, this seems unlikely due to the costs to the fungi. The colonization of leaves is accompanied by at least two costs to the fungi. Firstly, the colonization of live plant tissues requires specialized chemical mechanisms which allow for the invasion of living plant tissues and the evasion of their defenses. This would require both selection for the evolution of these mechanisms, as well as investment of resources to their construction during development. In other words, it is not a straight forward task that can be accomplished haphazardly and it is fundamentally different process than those used to colonize saprotrophic substrates such dead plant materials or wood (Kusari et al. 2012). In addition, if leaf colonization was a dead-end, achieving it would directly reduce the likelihood of colonizing more beneficial substrates since leaves would act as nothing more than a spore sink.

George Carroll's foraging ascomycete hypothesis (referred to hereafter as "FAH" [1999, Thomas and Vandegrift et al. 2016, Thomas et al. 2016]) proposes that the function of leaf endophytism may be to increase the success of dispersal to other substrates. While saprotrophic endophytes rarely fruit directly from leaves, the FAH proposes that after leaves senesce and fall, the endophytes are capable of colonizing saprotrophic substrates that they settle on in the environment. Thus, endophytism is described as a facultative life stage that bridges stages of

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saprotrophism. The benefit is that, during times of environmental scarcity, endophytes may have an increased likelihood of survival compared to spores or saprobic mycelia. This is because the highly buffered environment of leaves provides a water and food source to their fungal symbiont, regardless of surrounding environmental conditions (Thomas and Vandegrift et al. 2016). Probably the most important factor is water, since fungi heavily depend on the presence of moisture for survival, growth, and spore germination (Moore 1986, Eveling et al. 1990).

The foraging strategy can be viewed as a dispersal method that is an alternative (or modification) to spore dispersal. The effectiveness of spore dispersal has often been assumed to be virtually unlimited (Becking 1934, Fenchel and Finlay 2004). However, spore dispersal does have limitations. For example, it has been found that the vast majority of spores come to rest within a short distance of fruiting bodies, which imposes a spatial limit on their effectiveness (Galante 2011, Norros et al. 2012, Hussein et al. 2103, Peay and Bruns 2014). Also, spores have limited viability periods and are vulnerable to desiccation, especially in dry conditions.

The investment of spores into leaf colonization is essentially a form of bet-hedging, which is defined as a strategy that "reduces the temporal variance in fitness at the expense of a lowered arithmetic mean fitness" (Ripa et al., 2010). Direct spore dispersal by itself may result in a higher mean success rate in colonizing substrates, but it will be highly variable depending on environmental conditions. In other words, few spores will germinate during dry periods while many may germinate during wet periods. However, when a subset of spores from each sporulation event become endophytes, they decrease this variance of dispersal success. This is because they improve the success of sporulation events which occur in dry conditions.

The FAH has implications on the temporal dynamics of dispersal: while spores are released in high density bursts with a short duration, leaf-endophytes are released

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asynchronously in low densities over longer periods of time (Thomas et al. 2016). The latter simply reflects the life cycle of the tropical leaves, which can persist on trees for several years before senescence, and are shed without regard to seasonal timing. Presumably, the leaves are also shed independently of environmental conditions and so there is no assurance that endophytes will have increased dispersal success compared to spores; rather the gradual release of fungal propagules simply lowers the variability of dispersal success through time, and in doing so reduces the vulnerability of fungi to environmental variations.

To encompass the processes described above, I introduce a new term, *viaphytes*, for referring to fungi that undergo the lifestyle shifts described by the FAH. The reasons are that (1) referring to taxa as "foragers" is vague and could lead to confusion, and (2) referring to them as "foraging ascomycetes" is somewhat unwieldy and slightly inaccurate, since a subset of basidiomycetous endophytes likely possess foraging abilities as well. "Viaphyte" joins the word, *via* --defined as "travelling through a place en route to a destination (Via n.d.)"—with the suffix, *phyte*, which denotes a plant. Generally, the term will refer to fungi that colonize living leaves as endophytes and use the leaves to transfer to another substrate when the leaves dehisce. Within this study, I often use the term "viaphyte" more narrowly to refer to fungi which display an ability to transfer from endophytic leaf-tissue to a separate saprotrophic substrate.

For the FAH to be feasible and viaphytism to occur, it must be shown that this transfer from leaves to another substrate is possible. Thomas and Vandegrift et al. (2016) observed this transfer but they restricted the scope of their work to a single fungal genus, *Xylaria*. Therefore, it is unclear how prevalent this ability is among other fungal endophytes. Here I set out to conduct a more inclusive survey of the viaphytic abilities of endophytes present in tropical leaves, and I predicted that at least several species of endophytes would demonstrate an ability to transfer.

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In the event that this prediction was correct, I was also interested in assessing (1) the overall diversity of viaphytes, (2) how populations of viaphytes vary between leaves, and (3) the ecological roles of each of the viaphytic fungi.

Leaf endophytes are very diverse and have a wide taxonomic breadth, consisting of multiple phyla and numerous orders. As a subset of the endophyte community, I expected that viaphytes would also represent a wide taxonomic breadth. However, since the FAH predicts that dispersal via endophytism would benefit saprotrophic fungi in particular, I predicted that the majority of viaphytes would be taxa described as saprotrophs.

Materials and Methods

Culturing Methods

Culturing methods and are summarized in Figure 1. Twelve evergreen leaves of a randomly selected tree (*Nectandra lineatifolia* (Ruiz & Pav.) Mez) were collected in an Ecuadorian cloud forest. The tree was within Reserva Los Cedros, which is on the western slope of the Andes in northwestern Ecuador (00°18031.000 N, 78°46044.600 W), at 1000-2700m above sea level. Then, eight 2-cm^2 sections were cut from each leaf and surface-sterilized by successive immersion in 70 percent ethanol for one min, 5% sodium hypochlorite (equivalent to full strength bleach) for two min, then rinsed thoroughly in sterile water. The sterilization process ensured that no epiphytic spores or mycelium could colonize the wood substrate.

Eight 2 cm^2 sections were cut from each surface-sterilized leaf and placed onto twice-autoclaved white birch tongue depressors (Puritan, Guilford, Maine, U.S.A.) as a standardized angiosperm woody substrate. The eight sections from each leaf were split between two tongue depressors (4 sections each) resulting in a total of 24 tongue depressors. These were split evenly

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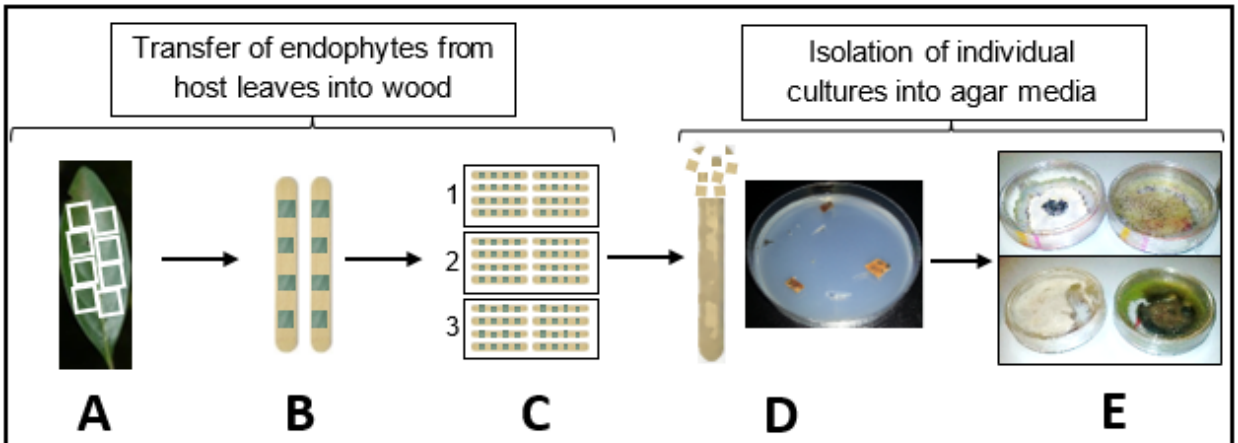

Figure 1. Viaphyte culturing process: **(A)** Twelve leaves of *Nectandra lineatifolia* were surface-sterilized and eight sections were cut from each leaf. **(B)** Leaf-sections were placed on sterile birch wood sticks, 4 leaf-sections per stick, resulting in a total of 24 sticks. **(C)** The leaves and birch sticks were incubated in three separate boxes for 6 weeks. **(D)** The sticks were broken into small fragments and placed on water agar plates. **(E)** Each distinct fungal colony in the water agar was excised and transferred onto a malt agar plate (each of these was an "isolate"). After all of the isolates matured, they were grouped together into common "morphotypes" (not pictured).

between three EtOH-sterilized Ziploc storage boxes and were incubated at the field station at room temperature for six weeks. Each box contained an open container of sterilized water to maintain humidity. The incubation allowed for the endophytic fungi to emerge from the leaves and transfer to the wood. After the inoculation period, the sticks were placed into airtight bags and brought to Dr. Bitty Roy's lab at the University of Oregon. Dr. Roo Vandegrift, PhD., carried out these initial steps at the field site in Ecuador, and the resulting inoculated wood is the same material that *Xylaria* viaphytes were isolated from (Thomas and Vandegrift et al. 2016).

At the UO lab, I isolated fungal culture strains from the inoculated wood by breaking approximately 15 small fragments (~5 mm^2 each) of wood from each tongue depressor and dispersing them evenly among five 100 mm water agar plates. As hyphae grew into the agar, small hyphal fragments were excised from growing ends using a dissecting microscope and a scalpel. Since many hyphal groups were growing in the same dish, care was taken to excise

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regions of each that appeared to be away from others so that only one culture was excised. These hyphae were transferred onto nutrient plates (MEA, 2% maltose). After a period of growth (seven or more days) the isolates were grouped into "unique taxa" based on macro- and microscopic features, each of which was assigned an arbitrary morphotype number. If numerous isolates appeared to belong to the same genus they were assigned the same morphotypes, even if they were apparently different species.

Identification of Viaphytes

DNA was extracted from a representative culture of each morphotype and the ITS region (the standard "barcode" gene for fungi [Dentinger et al., 2016]) was amplified using the fungal-specific primer set ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). The amplified sequences were sent to an external lab (Functional Biosciences, Madison, WI) for sequencing.

The ITS sequence data was edited using Geneious (v6.0.3; Biomatters Limited, Auckland, New Zealand), which was used to trim the low-quality portions at the ends and to generate consensus sequences from the two ITS reads from each morphotype. The consensus sequences were then compared to published sequences in the UNITE database (Abarenkov et al., 2010) using the *assign_taxonomy.py* function from the Quantitative Insights into Microbial Ecology (QIIME) pipeline (Caporaso et al., 2010). The UNITE database is a curated form of the GENBANK database that excludes low quality and non-informative reads of fungi (Koljalg et al., 2013). I further curated the UNITE database by removing all sequences that did not have a taxonomic identity to at least the genus level. These taxonomic assignments were used in all downstream statistical analyses.

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Species richness was estimated using Chao2 and Jacknife1 estimators (Burnham & Overton 1978, Chao 1984, Colwell & Coddington 1994). Diversity was estimated between all leaves, within leaves, and within boxes using Shannon's index (log base *e* was used; Shannon, 1948) and Simpson's index (1-*D*; Simpson, 1949). Sampling effort was visualized with species accumulation curves. Data were analyzed using R Statistical Software, v. 3.1.0 (R Core Team 2014), including the *vegan* package (Oksanen et al. 2013).

Trophic modes were assigned to each genus by using the FUNGuild online tool (Nguyen et al., 2016). Any genera that were not assigned a guild through this tool were assigned one based on the taxonomic literature.

Results

Diversity and Abundance of Viaphytes

Numerous endophytes were able to transfer from leaves into wood. 477 separate fungi cultures were isolated from wood after making the initial transfer from leaves to wood. These were segregated into 67 distinct morphotypes, 62 of which were successfully identified (59 by DNA, 3 by morphology). DNA identification resulted in the consolidation of the morphotypes into 26 unique OTUs (21 identified to the species level and five identified to the genus level [*E-score*=0]). The number of isolates represented by each taxon was unequal, with 57% of the isolates represented by just 2 genera (*Trichoderma* and *Penicillium*) while 38 of the morphotypes were isolated only once (Figure 2). The mean number of unique OTUs within each leaf was 5.67 (*s*=2.1) and the minimum number of OTUs for any leaf was four (Table 1).

The species accumulation curve (Figure 3) was non-asymptotic, indicating that the full presence of viaphytes was not isolated. Estimates of actual species richness ranged from 39.4

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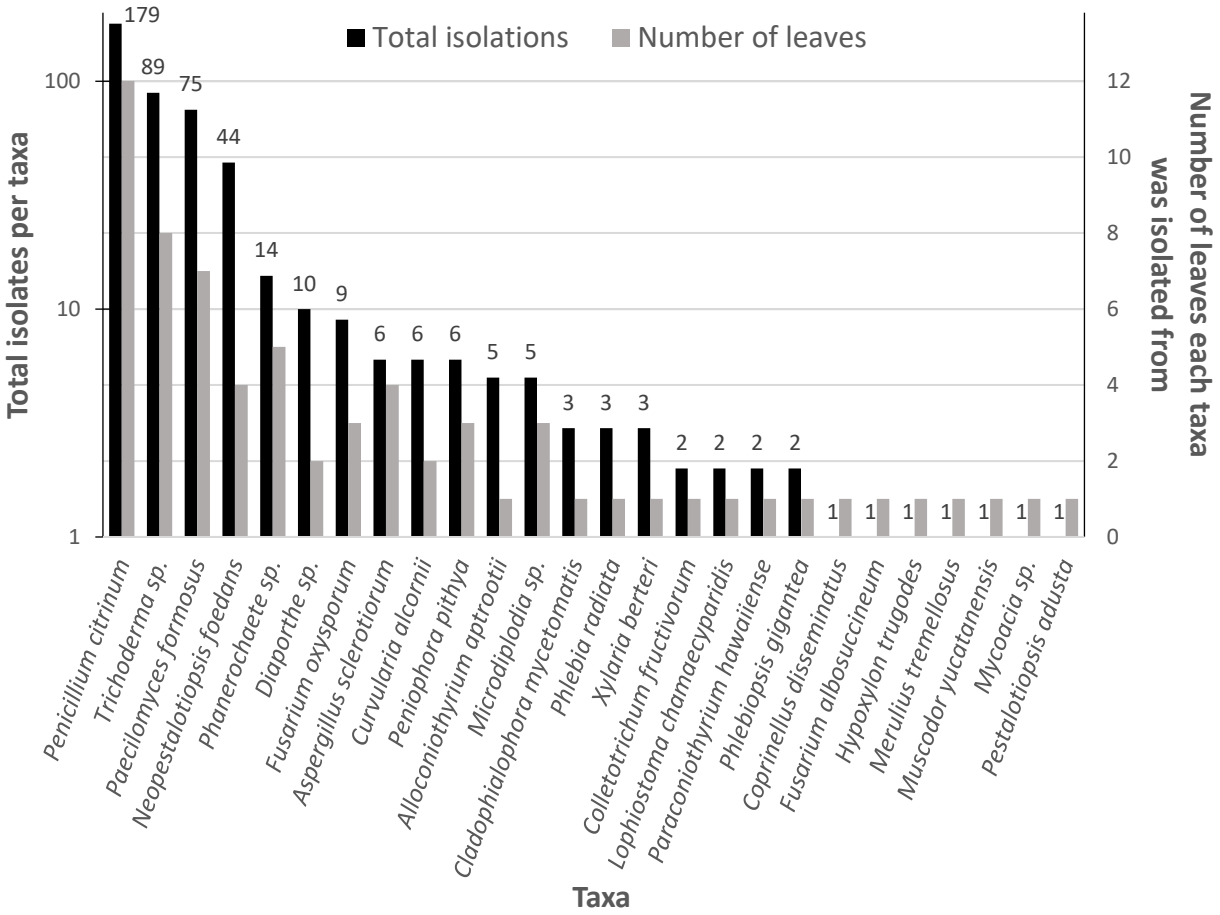

Figure 2. Summary of identified fungal endophytes that transferred from host leaves into a woody substrate. From 12 leaves, 26 taxa transferred to wood and were subsequently isolated. Of a total of 472 identified isolates, 82% were represented by the four most common taxa. The total isolates per taxa roughly corresponds to the number of leaves they were isolated from. The numbers on the bars specify the number of cultures per taxa. [Note: the left axis is on a logarithmic scale.] 5 isolates remained unidentified and are not included in the figure.

(first order jackknife; *SE*= 4.7) to 57.3 (chao2; *SE*=25.3).

Viaphyte communities within boxes were similar to each other (PerMANOVA: F (1, 23) = 6.54, p = 0.001) while communities between stick pairs from the same leaf were not more similar to each other than to other sticks. (PerMANOVA: F (1, 23) = 1.05, p = 0.350; Figure 4). Isolates of the four most common taxa were concentrated in single boxes, with 100% of

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Table 1

Measured and Estimated Diversity Values

	OTU Richness	Shannon's index	Simpson's index (1-D)
Measured diversity			
Leaves (*N*=12)			
Min.	4	0.41	0.18
Max.	11	1.91	0.8
Standard dev.	2.1	0.46	0.21
Mean	5.67	1.12	0.55
Boxes (*N*=3)			
1	18	1.86	0.76
2	10	1.28	0.61
3	13	1.06	0.42
Standard dev.	4.04	0.41	0.17
Mean	13.67	1.4	0.6
Total measured diversity	26	1.99	0.78

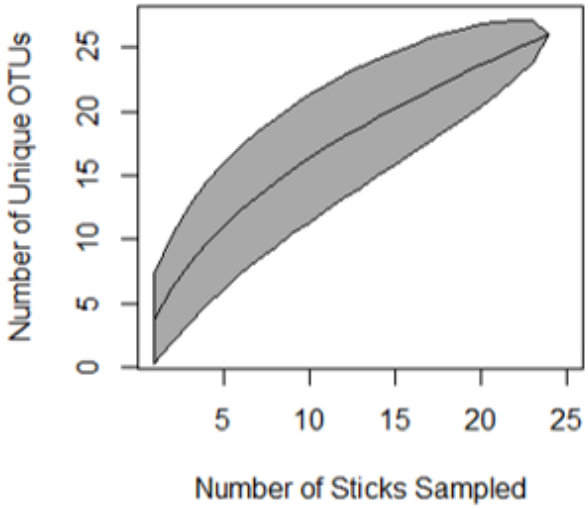

Figure 3. Species accumulation curve for viaphytes. The culturing did not achieve a saturation of viaphyte OTUs.

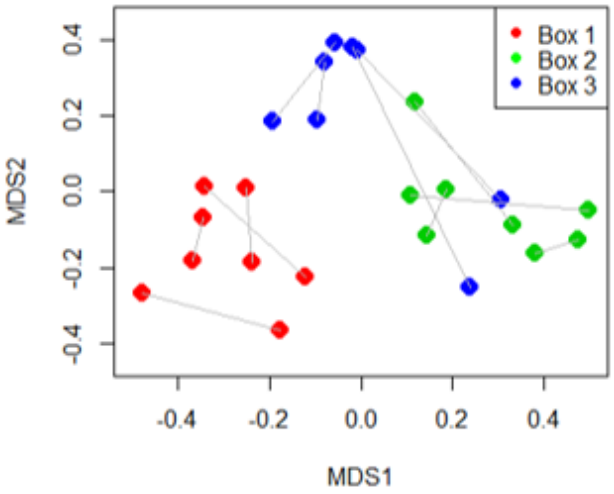

Figure 4. NMDS of viaphyte communities. Each dot represents an individual stick. Lines connect sticks that were inoculated by the same leaf and coloring is done by box.

Neopestalotiopsis foedans in box 1 (*N*=44), 96% *Paecilomyces formosus* in box 1(*N*=75), 87% *Trichoderma* in box 2 (*N*=89), and 61% of *Penicillium citrinum* in box 3 (*N*=179). In all of the cases except *Penicillium citrinum*, the most populace boxes were the only boxes which had all 4

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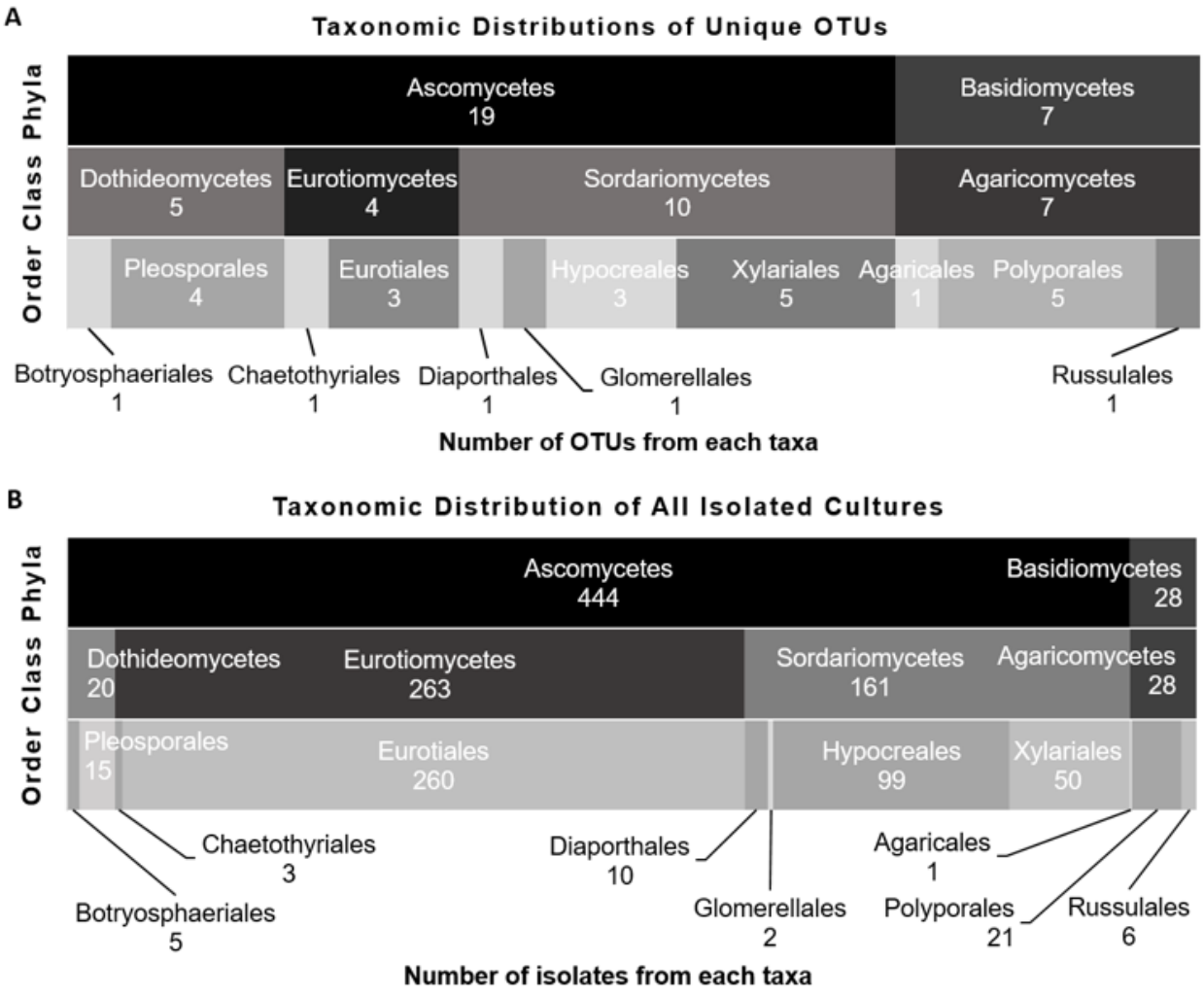

Figure 5. Taxonomic distributions within the phyla, class, and order levels as a function of **(A)** unique OTUs and **(B)** total number of cultures isolated. Each row of unique OTUs totals to 26. Each row of isolated cultures equals 477. The vertical arrangement of taxa reflects the nested hierarchy of the taxonomic groups (e.g., Botryosphaeriales are in the Dothidiomycetes class and the Ascomycetes order).

leaves colonized by the respective taxa, while *P. citrinum* was found on all sticks across all boxes.

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Taxonomic Distribution

The higher order taxonomic ranks in our samples included two phyla, four classes, eleven orders, and sixteen families (phyla, class and order proportions are displayed in Figure 5). Ascomycetes and Basidiomycetes were present, though Ascomycetes were the dominant phyla both in terms of OTUs and in total isolates (73% and 94%, respectively). Sordariomycetes were the most common class in terms of OTUs (38.4%), while Eurotiomycetes had the most isolates (55.7%). Xylariales and Polyporales were the most common orders in terms of OTUs (19.2 % each), while Eurotiales had the most isolates (55.1%).

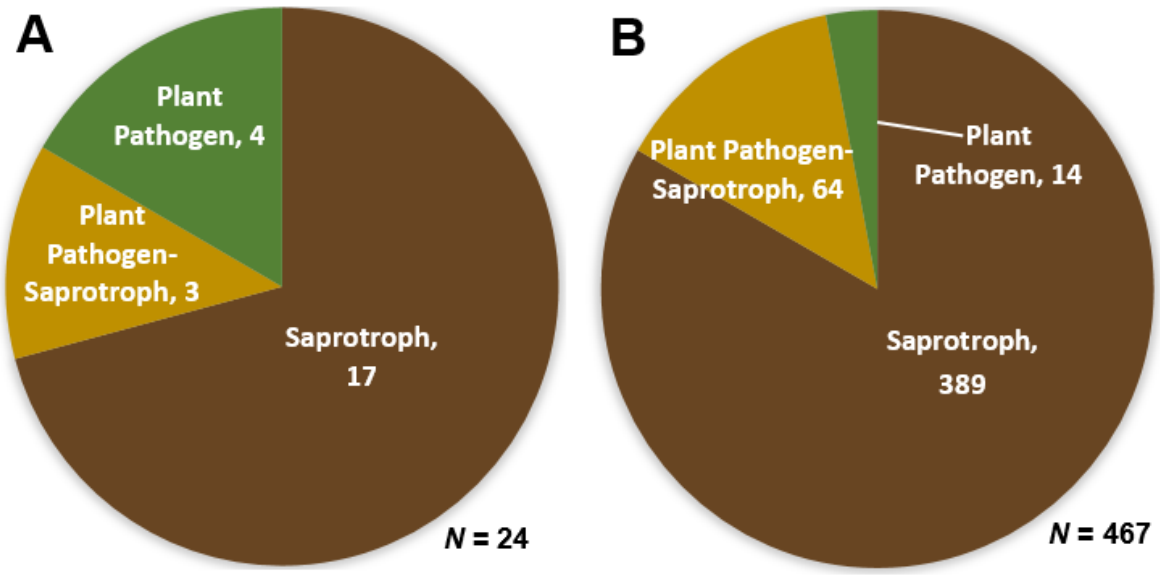

Figure 6. Trophic modes of viaphtyes by **(A)** number of genera and by **(B)** number of isolates.

Trophic Modes

The FUNGuild database assigned a trophic mode to all but two of the genera of viaphytes. The first non-assigned genus, *Alloconiothyrium*, is a newly described genus presently represented by a single species, *A. aptrootii*, which was isolated from a soil sample in Papua New Guinea (Verkley et al. 2014). I therefore did not assign it a trophic mode since so little

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information is available on it. The second, *Neopestalotiopsis*, I classified as a "plant pathogen/ saprotroph" based on substrates listed in species descriptions (Maharachchikumbura et al. 2014).

The viaphyte genera of our study fit into three different trophic modes: *saprotroph*, *plant pathogen*, and *plant pathogen*/*saprotroph*. Saprotrophism was the dominant trophic mode for viaphytes. Saprotrophs had the highest proportion in terms of number of genera (70.8 %; 17 out of 24) and in terms of number of isolates (82.3 %, 389 out of 467; Figure 6). Four of the genera were plant pathogens (16.7 %) and three were plant pathogen/saprotrophs (12.5 %). Of the isolates, 64 were plant pathogen/saprotrophs (13.7 %) and fourteen were plant pathogens (3.0%).

Discussion

Viaphyte Prevalence

In this culturing study, I isolated 477 viaphytes comprising 26 different taxa. This demonstrates for the first time that a wide array of tropical leaf endophytes can colonize separate woody substrates, and hence can alternate between an endophytic and a saprotrophic life stage. The host leaves contained between four and eleven viaphytes each, showing that multiple fungal species have a potential for viaphytic dispersal from within each leaf. This high frequency of transfer not only shows that viaphytic dispersal of saprobic fungi is possible, but that it is probably mechanistically straightforward.

While our present viaphyte survey looked solely at one individual tree, it seems unlikely that this host is unique in allowing the transfer of endophytes to other substrates, or that the viaphytes observed within its tissues are only able to transfer from this particular host. In other words, if the host is taken to represent a typical broad-leaved tropical tree, and the fungi it hosts are similar to endophyte communities in other tropical leaves (Rodriguez et al. 2009), it follows that viaphytes are likely commonplace symbionts in the leaves of tropical forests.

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Even if fungi with viaphytic abilities are common, it is not clear how often viaphytic life-stage transfers actually occur in natural systems. While endophytes in this study were placed on sterile wood substrates, in nature viaphytes would face competition from other sources of colonization, such as spores or latent saprotrophs already present in the wood. It is likely that each of these colonization mechanisms each have their own set of virtues. For instance, it is possible that foliar endophytes have some advantages, at least over spores, that are conferred by their leaf hosts since leaves provide a source of water and carbon to colonizing fungi. In addition, leaves could trap rain water or dew between the leaf and substrate and may even act as barriers that exclude spores from being deposited on the woody substrate directly beneath the leaf. Spores probably have their own sets of advantages over viaphytes. For one, spore dispersal may be favored due to it reduced complexity (*i.e.*, successful viaphytes must accomplish two independent colonization events (first of leaf, then of wood), while spore dispersal requires just a single colonization such as abundance. Also, spores certainly outnumber the number viaphyte-bearing leaves. Finally, spores have a considerably greater maximum travel distance compared to viaphytes, even if these distances are achieved by only a small subset of total spores. The relative successes of different dispersal methods are likely determined by a complex array of factors, and future studies should be designed which address these dynamics.

Another question is, if viaphytism occurs in nature, how often does it impart the benefits described by the FAH? This question has only been directly confronted by Thomas and Vandegrift et al. (2016), who found signs that viaphytism is having measurable effects on fungi spatial ecology. In particular, they found that wood-borne fruiting structures (*i.e.*, stromata) of some fungal species had spatial ranges that were confined around stream areas, while endophytes of the same species had spatial distributions that extended away from streams. This is consistent

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with the FAH prediction that endophytes will have higher tolerance of dry conditions afforded by the highly buffered environment of the leaves.

Thomas and Vandegrift et al. (2016) have conducted the only study to date which directly tests the benefits of the FAH *in situ.* While it addresses the spatial dynamics of the endophyte-saprotroph duality, the FAH also predicts temporal effects for viaphytes. In particular, the FAH predicts that endophytes will survive dry periods better than saprotrophic forms. Hence, when conditions are too dry for stromata to form, spore release will not occur and viaphytes may become the primary source of inoculum. In areas that undergo prolonged dry periods, it is conceivable that fungi which lack viaphytic capabilities may be locally excluded. This rests on the assumption that viaphytes in leaves can persist longer than not only spores, but also sclerotia (dormant mycelial structures) and saprobic mycelia. If this speculative scenario is accurate, it means that temporal effects of viaphytism could affect species ranges at landscape or ecosystem scales. Future studies should therefore address the temporal effects of viaphytism.

Another effect of viaphytism is that it could increase outcrossing events by reducing the chances of mating between spores of the same parent. Spores released from the same fruiting event have a likelihood of colonizing the same nearby substrates. However, if a subset of those spores delay their colonization of wood by becoming viaphytes, then they increase their chances of mating with a less-related fungal colony that was initiated from spores of a different parent.

The richness of viaphytes we isolated is an underrepresentation of the true richness according to the species accumulation curve (Fig. 3). In fact, the total estimated richness produced by jackknife and chao2 calculators (N=39 and 57, respectively) are also probably low, because they rely on the assumption that all of the species within the samples (leaf sections in this case) are capable of being detected (Schmit and Lodge, 2005). In fact, there are a number of

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taxa that are incapable of growing in culture and therefore will never be detected at any level of culturing effort (Schmit and Lodge, 2005). There are additional reasons that the diversity was likely underestimated. Firstly, culture-dependent studies favor the isolation of fast-growing species. Secondly, a handful of the isolated morphotypes (N=3) *were* never identified due to both a lack of useful DNA and useful morphological features for identification, and these taxa were not included in the species accumulation curve data. Thirdly, since morphotypes were delineated roughly at the genus level, and DNA was analyzed of only one isolate from each morphotype, our design inherently overlooked the richness of viaphytes present at the species level. For example, in our *Penicillium* isolates there was a wide range of morphologies present which likely represented distinct species, but we only sequenced a single isolate from the *Penicillium* morphotype. In this way, morphotypes with multiple isolates may have represented multiple species which we missed. Finally, it is also possible some morphologically similar genera were missed by being falsely placed into the same morphogroup.

The viaphyte community was characterized by a few OTUs with high abundances and a large number of OTUs with low abundances (Fig. 2). While this pattern is typical among leaf endophyte studies (Arnold et al. 2000, Arnold et al. 2007, Gazis and Chaverri 2010), some patterns in the data suggest that they are partly due to methodological biases. For instance, *Penicillium spp.* and *Trichoderma spp.* were both observed to be fast growing in culture in this study, and culture-based studies are known to be biased for faster-growing taxa (Kirk et al. 2004). Also, the PerMANOVA results showed that sticks within boxes were more similar to each other than to sticks from other boxes. Yet, this can't be explained by the fact that sticks inoculated by the same leaves were housed in the same boxes, since, interestingly, these sticks were not significantly more similar to each other than they were to other sticks. The likely

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explanation is that the dominant taxa contaminated the sticks within their respective boxes via sporulation during the inoculation period. This is reflected by the result that each of the four most dominant taxa (*Penicillium, Trichoderma, Paecilomyces, and Neopestalotiopsis*) had a high proportion of isolates concentrated in one box, and these boxes were the only which had every stick colonized by those taxa. The most extreme case was with *Neopestalotiopsis*, for which 100% of the 44 isolates were spread among all eight sticks of a single box. This interpretation is further supported by the observation that all four of these dominant taxa readily produced a high quantity of conidia in culture. Therefore, the number of isolates for these abundant taxa should be interpreted with caution as they may not reflect the actual abundance in host leaves, but rather may be an artifact of rampant within-box contamination and relatively fast growth in culture.

Taxonomic Distribution

The viaphytes in this study belong to a wide taxonomic breadth, consisting of both Basidiomycetes and Ascomycetes. This implies that the benefits described by the FAH are available to Basidiomycetes as well. The taxonomic distribution of viaphytes from this study resemble those of general tropical leaf-endophytes described in other work. In particular, Arnold et al. (2007) reported the same three Ascomycete classes (Eurotiomycetes, Dothideomycetes, and Sordariomycetes), with similar proportions, although they did not report Basidiomycetes at all.

The wide taxonomic distribution of viaphytes suggests that viaphytic dispersal may be a deeply ancestral trait. This would parallel general endophytes, which appear to have associated with plants as long as 400 mya (Krings et al. 2007). Future taxonomic and paleontological work may help inform when viaphytism emerged within the fungi.

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Trophic Modes

The vast majority of viaphytic cultures and taxa from our study are classified as ecological saprotrophs, with 17 of the 24 genera classified as saprotrophs, and three more genera having some saprotrophic abilities. The prevailing explanation for the occurrence of saprotrophic fungi in endophyte communities has been that they are latent saprotrophs, waiting to use the host tissue as food source upon senescence (Persoh 2013). However, leaves often have endophyte communities that are dissimilar to those on adjacent woody tissue (Sun et al. 2011, Tateno et al. 2015). Furthermore, leaves do not provide an adequate food source for the development of fruiting structures for many saprotrophs (*e.g.*, wood decomposers). Also, Unterseher et al. (2013) found that OTUs in leaf endophyte communities were different from OTUs found in dead branches attached to the same trees. Therefore, there is evidence that the latent-saprotroph argument does not work for all saprotrophs that are found as leaf-endophytes.

Conclusion

As an alternative to the latent-saprotroph hypothesis, the FAH suggests that many saprotrophs use endophytism to modify dispersal to their primary (*i.e.* reproductive) substrates. Here I demonstrate for the first time that a diverse assemblage of foliar endophytes can directly colonize woody substrates from leaves, and that a high proportion of these fungi are ecological saprotrophs. This work provides new support for the FAH. While the prevalence of viaphytic dispersal in nature is unresolved, the diversity and abundance of viaphytes described here suggests that it may be commonplace. If this is the case, viaphytic dispersal may be affecting several aspects of fungal ecology such as dispersal, drought tolerance, geographic distribution in significant ways. These dynamics are largely unexplored and represent a vast potential for future

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research.

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