COMMON GREENBRIER (*SMILAX ROTUNDIFOLIA* L.) AS A MODEL FOR UNDERSTANDING FUNGAL COMMUNITY ORGANIZATION IN THE PHYLLOSPHERE

by

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ABSTRACT OF THE DISSERTATION

Common greenbrier (*Smilax rotundifolia* L.) as a model for understanding fungal community organization in the phyllosphere

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Fungi asymptomatically infect all terrestrial vegetation, but the structure and assembly of these fungal communities are poorly understood. *Smilax rotundifolia*, a common woody vine of the northeastern United States, was used as a model to study endophytic (internal colonizing) and epiphytic (surface colonizing) fungal communities, from the perspectives of niche-based influences, spatial variation, and evolutionary relationships. Wild greenbrier plants were sampled in New Jersey (USA) in late winter at a single site, and again in mid-summer, in a multi-site, multi-tissue, sampling effort.

Fungal communities of the plant surface and interior were made up of mostly different species. Correlative relationships were found between some fungal species' abundances, but none were seen between species that were strongly restricted to the surface and those strongly restricted to the interior. The summer, multi-site study, revealed that the strongest factor determining fungal community composition was surface vs. interior habitat, followed by tissue/organ type, and lastly general geographic location. The effect of season was also studied by comparing the single-site winter dataset against the data from the same site sampled in summer. Season had a major influence on some

fungal species but not others, similar to influence of tissue type. Also, in a very novel finding, it was found that certain endophytes showed statistically different abundances, depending on the distance from the stem base at which a sample was taken. The optimum height differed for different fungal species. At the scale of a single stand of plants, it was found that epiphytic, but not endophytic, samples showed a significant correlation between spatial proximity and fungal community similarity. Finally, the phylogenetic relationships were studied between congeneric fungal species that were common on the plant. Two-gene phylogenies were constructed using *Smilax*-derived isolates, along with downloaded sequences of well-defined species in the same genera. These congeneric species were found to be only distantly related (i.e., they were widely separated within the known phylogenies of their genus). The final chapter is a literature review, bringing insight from the present dissertation research to identify important unanswered questions. Evidence for the role of plant secondary metabolites on endophytic fungi is discussed.

DEDICATION

This dissertation is dedicated to the memory of my grandfather, Joseph T. Judge, who shared and encouraged my interest in the natural world.

ACKNOWLEDGEMENTS

I am very grateful to my wife Josephine for her support and companionship throughout my studies, and especially for her intelligence and hard work in editing my manuscripts.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter I. Introduction

Most of the research and data presented in this dissertation can be thought of as a detailed mapping of the community of filamentous fungi associated with the common North American forest vine, *Smilax rotundifolia*. In other words, it is a visualization, from various perspectives, of the invisible microbial community, in order to gain insight into the processes that structure these communities, and that probably structure microbial communities in other plants and perhaps other climates as well. However, the picture generated is not an inevitable result of the plant studied. As is typical of microbiology, there were many subjective decisions about how and where to make observations and about how to analyze the data that went into making the final product. The primary perspectives from which these micro-communities were mapped were **spatial** (at both a local and geographic scale), **niche-based** (including surface vs. interior, tissue type, season, stem location), and **evolutionary** (relation among dominant species in the same genus). The immediate practical need for this knowledge may seem obscure. However, diverse communities of fungi asymptomatically infect all of the terrestrial plants that surround us, in both natural and agricultural, or otherwise managed, landscapes. The lack of understanding of the impact of these communities, their positive or negative effects, and their basic ecology is a blind spot in ecology and basic biological understanding of our surroundings. The accurate visualization, and recognition of biological patterns, is a foundational step in building an ecological understanding of any biological community (for example, the recognition of elevational and latitudinal patterns in terrestrial vegetation over two centuries ago; see Von Humboldt and Bonpland 2009, with introductory notes by S.T. Jackson). While the field of endophytic ecology is certainly

beyond the phase of simple species survey and/or collection, there is a need for more researchers to quantify and identify patterns in phyllosphere fungal communities, and to ask the question of how these communities are formed. This could lead in time to a better practical ability to influence and understand the phyllosphere fungi. The process of generating this research, combined with analysis of past research in the field, has, I believe, spurred many potentially fruitful hypotheses and questions that may lead to significant advances in the field, outlined in the final chapter (**Ch. V**).

In addition to the other questions explored, all of the lab or field work here focuses on surface (epiphytic) and interior (endophytic) fungal communities of *Smilax rotundifolia*. One consistent question addressed in this dissertation was how epiphytic and endophytic fungi differ from each other. For those who study endophytic fungi (often with a view of biodiversity exploration) there is a perception that epiphytic fungal communities consist of a few generalist species and are uninteresting. Throughout this work, I put this assumption to the test, while at the same time asking questions about endophytic communities, and exploring the fungal biodiversity of a plant species and genus that has not been studied from the endophytic perspective in the Americas.

In **Chapter II**, I introduce the plant *S. rotundifolia*, its ecology as a common and versatile weed, its biomass, its importance to wildlife, insect associations, and the peculiar *Smilax* lineage from an ecological/phylogenetic perspective - woody monocots of many climates and geographic regions. This short chapter combines some literature review on the plant along with my own miscellaneous observations that might relate to its use as a fungal substrate. Although many of the questions raised in the dissertation were

not specific to *Smilax,* it is a prominent and interesting plant in the flora of North America. The cataloguing of the plant's phyllosphere mycobiota is a specific contribution to the documentation of North American wildlife and natural history.

In **Chapter III**, I first address the question of how surface and endophytic communities differ in both species composition and structure. I used a simple, but labor intensive, procedure to sample stem surfaces by taking tape lifts, then finely cutting up the tape and plating it onto nutrient media (endophytes were sampled in the traditional way using surface sterilization, then tissue plating). Surprisingly, this tape lift method has not been used in previous studies comparing endophytes to epiphytes; it should give the most accurate comparison of the two micro-habitats compared to any previous studies in the literature (as discussed in the chapter). I began this study originally with an attitude of skepticism towards the idea that endophytes were a separate and important group from epiphytes. I was skeptical because I could observe dark masses of mycelium on *Smilax* surfaces (see Ch. II), while at the same time I had great difficulty in definitively observing any endophytic hyphae via microscopy and staining methods. The results, and further literature review, put this skepticism to rest and reinforce the concept of endophytism as a specialized niche.

The other major goal in this study was to determine how endophytes and epiphytes are spatially distributed across a a forest stand of greenbrier, of about 200 meters at its longest. In plain terms: are micro-fungi well dispersed as if they were evenly, mechanically sprayed across the entire site, or are they clustered in certain areas, rare in others, which would seem to indicate a slow, or imperfect, local dispersal.

Theoreticians have come to multiple conclusions about the implications of imperfect local dispersal, or different efficiencies of dispersal, with the conclusions depending on whether niche and neutral models are used as well as some other parameters to the models. (Chave et al. 2002, Ai et al. 2012). My results indicated that there is spatial correlation in epiphytic communities, probably reflecting imperfect dispersal of propagules within the site. While endophytic communities did not show spatial correlation, I interpret the results to mean that imperfect dispersal is probably also present in endophytic communities since the types of fungi involved are similar, but the dynamics of the endophytic community override the spatial signal. (see Ch. III discussion). The difficulty in interpretation lies in the fact that dispersal was not directly measured, but instead the spatial correlation of communities which is the end result of immigration (fungi coming in from other sites, or other plants), local dispersal ability, and ability of colonies to survive (environmental adaptation) the environment and possibly even competitive or facilitative interactions. I suggest that the endophytic colonies, if in their proper environmental niche, are more stable, so that longer colony residence time leads to more homogeneous, less spatially correlated communities over time compared to the epiphytic communities. This may be indicative of slightly stronger niche dynamics among endophytes compared to the epiphytes, where the different species ability to survive may be more equivalent (neutral dynamics).

Finally, furthering the case for strong niche dynamics in the endophytic community, this study revealed a very striking pattern of niche partitioning in stems of greenbrier by endophytes, which appeared to partition by stem height. This was not an expected or planned result and unfortunately was not recognized until a long time after

the initial data analysis, so that there was no time for sufficient experimental follow up studies. I had taken 10 samples from every stem in a systematic way, at regular intervals from the base and the tip of the stems. This was done mainly to characterize fungal communities of each stem for the spatial aspect of the study. There was also a plan to explore successional dynamics of young versus older stem portions, but after realizing that greenbrier vines extend very quickly in the spring, so that the top and base of vines are often virtually the same age, I was not anxious to analyze the effect of stem position. When I finally did the analysis, it became clear that several individual endophytic species showed a profound preference, beyond the level of just base vs. tip, but for certain regions of stems at different distances from the stem base (Fig. III-6, III-7). The meaning of this pattern is still open to interpretation, since I did not have time to follow up on it experimentally, but I suspect it is related to internal plant chemistry, and discuss this further in both Ch. III and in Ch. V.

The next sampling effort, described in **Chapter IV,** was motivated in response to observations made in the Ch. III study, that many unidentified coelomycetes were found to be important components of the epiphytic fungal community of greenbrier stems (also see Appendix G, Fig. A-7). This did not fit the common perception of epiphytes as being ubiquitous 'weedy' hyphomycetes. Since these epiphytes did not appear to be all ubiquitous species, a follow up study was planned to expand sampling to multiple environments across the region, and determine if certain epiphytes were universally associated with *Smilax* (within that range), or if the coelomycetes of the plant surface might be incidental to the larger surrounding plant community. By sampling in different environments, I could determine if the epiphytic community was largely determined by

factors extraneous to the greenbrier plants. At the same time, the study took place in a different season, summer, allowing seasonal comparison to the previous study, and leaves were also present, allowing for tissue comparison. The results of this study showed that surface vs. interior micro-habitat was the most important aspect of fungal niche determining the community composition, followed in importance by tissue type, while location was found to have a relatively small effect. Season could not be directly compared to these other factors, but also was shown to have a statistically significant, and not subtle, effect on species composition. The overall impression of the study was of the importance of micro-niche over general environment.

Certain *Pestalotiopsis* species were found to be strongly associated with greenbrier wherever it was sampled (see Appendix G, Fig. A-9). Future taxonomic and sampling work will be needed to determine if any of these are host specific. However, the strong *Smilax* host affinity shown by some species suggests possible host specialization in epiphytes. If this is the case, then epiphytic diversity, like endophytic, may be an important component of earth's biodiversity.

Finally, from the evolutionary perspective, an interesting aspect of greenbrier phyllosphere fungi was that in several cases, there were several co-dominant morphotypes in the same genus. This allowed for us to ask the question of how these species were related to each other. Were they derived from a common ancestor that specialized on greenbrier, then split somehow into two or more species? Or were they derived from completely different parts of the genus family tree, coming to exploit the plant with different adaptations to begin with? The answer appears to be the latter.

Evolutionary aspects of fungal community structure are detailed in **Ch. V, section 5**, and in **Appendix D**.

Besides presenting the results of original research, this dissertation also contains a synthesis chapter, **Chapter V**. This chapter covers the literature review aspect of the dissertation, in addition to Chapter III and IV, which are both written in the format of stand alone research papers. The goal is to outline what is known of endophytic biology so that we can build an intellectual model of how an endophytic community forms. After outlining the different categories of endophytes, known life histories, and nutritional strategies of endophytes, this chapter attempts to identify major unanswered questions in phyllosphere community ecology and describe tractable ways to explore these. The emphasis is on endophytes rather than epiphytic fungi, mostly due to the large body literature that must be encompassed. Towards the end this chapter reviews what little is known about the relationship between plant secondary metabolites and fungal endophyte colonization, as well as covering some aspects of latent infection from the pathology literature, that are relevant to the study of asymptomatic fungal communities.

Chapter II. Background and observations on the plant *Smilax rotundifolia*, or common greenbrier

Smilax in the flora of North America and the world

S. rotundifolia is a woody, perennial vine having thin green stems (<1 cm diameter) armed with broad-based prickles (Gleason and Cronquist 1991). It is often found both scattered and forming dense thickets that spread via underground stems (Smith 1974). In the ecological manuals of American woody vegetation (e.g. Goodrum 1977, Smith 1974), the plant is classified as a type of greenbrier, which simply means a member of the *Smilax* genus, and conveys its green and prickly character.

Recent phylogenetic studies suggest that *Smilax* should be considered the sole genus of the family Smilacaceae, a monocot family in the order Liliales (Qi et al. 2013). It is an unusual family among monocots in that venation is reticulate between the major veins (Holmes 2002). Besides reticulate venation, typical traits of the genus are climbing habit, paired petiolar tendrils, unisexual flowers, umbellate inflorescences, fleshy berries, and stoloniferous or tuberous rhizomes (Cameron and Fu 2006; Qi et al. 2013) - in all of which *S. rotundifolia* is representative. *S. rotundifolia* is distinguished from similar *Smilax* spp. by the combination of its shiny, roundish, heart shaped leaves, frequently four sided stems, broad prickles, and black, frequently glaucous, berries.

Although the term *greenbrier* is generally understood to mean woody and prickly green vines, the genus itself is diverse, also containing species that are unarmed and/or herbaceous, and some even without tendrils. Species that are herbaceous (considered

Smilax sect. *Nemexia*) and exude a fragrance of decay are sometimes known commonly as "carrion-flower" (Rhoads and Block 2007). In the Northeastern United States there are 6 woody species of *Smilax* (*S. glauca* Walter, *S. hispida* Muhl., *S. bona-nox* L., *S. laurifolia* L., *S. rotundifolia* L., *S. walteri* Pursh.), and three herbaceous species (S. *pseudochina* L., *S. herbacea* L., *S. ecirrata* (Engelm.) S. Wats) (Gleason and Cronquist 1991). Across all of continental North America (excluding Mexico) there are 20 *Smilax* species (Holmes 2002), and worldwide the genus is estimated to contain about 210 species, distributed across both tropical and temperate regions in both hemispheres (Qi et al. 2013).

The natural range of *S. rotundifolia* is extensive, according to Gleason and Cronquist (1991) ranging from Nova Scotia to northern Florida on the east coast, and extending west to lower Ontario, Michigan, southeast Missouri, and eastern Oklahoma and Texas. Other sources (Goodrum 1977, USDA-NRCS 2015) include parts of Minnesota, South Dakota, and Kansas in the range. In the more northeastern parts of this area, it is the most common *Smilax* species (Gleason and Cronquist 1991). Because of this, most authorities call *S. rotundifolia* by the common name of "common greenbrier," (Goodrum 1977, Smith 1974, Holmes 2002), though others call it "roundleaf greenbrier," (USDA-NRCS 2015). In most of this dissertation, I refer to it economically as simply "greenbrier," since no other congeneric species were studied. Other common names include: catbrier, greenbrier, sawbrier, horsebrier, sowbrier, common bullbrier, bamboo-brier, biscuit-leaves, bread and butter, devil's hop vine, hungry vine, and wait-a-bit (Goodrum 1977, Smith 1974, Rhoads and Block 2007).

Growth, life-cycle, habitats

S. rotundifolia does not produce tubers like some *Smilax* species, but spreads via thin underground stems (Smith 1974). The plant is adept at growing in both sunlight and shade, being both a successional species and a persistent understory inhabitant (Smith 1974). As the plant grows most extensively in full sunlight, it may be that it often colonizes old fields or burned sites early via seed or rhizome, and then continues to persist in the understory. Most new growth of canes is completed within about 30-45 days in the spring (Smith 1974), and canes are said to persist for 2-4 years before dying back (Goodrum 1977). I have observed that a large mass of dead or dying canes is typically present in thickets of *S. rotundifolia* providing a steady resource for saprotrophic fungi. Another interesting aspect of tissue senescence in the plant involves the process of leaf fall. As noted by Coker (1944), there is no abscission layer at the base of the petioles that would allow the petiole to fall from the twig leaving a neat scar. Instead, the part of the petiole near the leaf base decomposes, allowing the tendrils attached to the base of the petiole to remain. *S. rotundifolia*'s leaves are tardily deciduous (Gleason and Cronquist 1991), and I have observed that many leaves show much decay and microbial damage while still alive and attached to the plant as the autumn and winter seasons progress. Depending on how sheltered a site is, many leaves may persist attached to the plant long into winter.

Smilax species can be a relatively large part of the understory and ground layer forest vegetation. For example, McEwan et al. (2005), sampled vegetation layers in 80 sites over a 52 ha old growth forest in the Appalachian regions of Eastern Kentucky. They found that *S. rotundifolia* was the most important of resident shrub-layer and

ground-layer species, having 1,209 stems/ha in the shrub-layer, and 4,938 stems/ha and highest cover, in the ground-layer. If *Smilax glauca* and *Smilax hispida* density are added in, the ground layer contained 6,688 *Smilax* stems/ha (*Acer rubrum* seedlings, considered transient, were the only species with higher density). In New Jersey, greenbrier is often widely scattered throughout forests or parks in separate stands, though in some pineland and coastal sites in the state, *S. rotundifolia* can also make up a tremendous component of ground-layer and shrub-layer vegetation (e.g., at the coastal forest of the Edwin G. Forsythe National Wildlife Refuge, in the far southern part of the state the plant is ubiquitous). For saprotrophic fungi, *S. rotundifolia* provides a steady source of dying and dead material, in the form of not only leaves, but constantly dying stems, tendrils, and petioles. The presence of many dead *Smilax* stems are a major component of its growth. For example (Ohman 2006), studying *S. rotundifolia*'s effect on fire in grasslands that had been invaded by the plant, assessed that 72% of the dry weight of fuel beds was dead material, primarily stems. The fact that most litter biomass is stem, not leaf, sets it apart from most other woody plants.

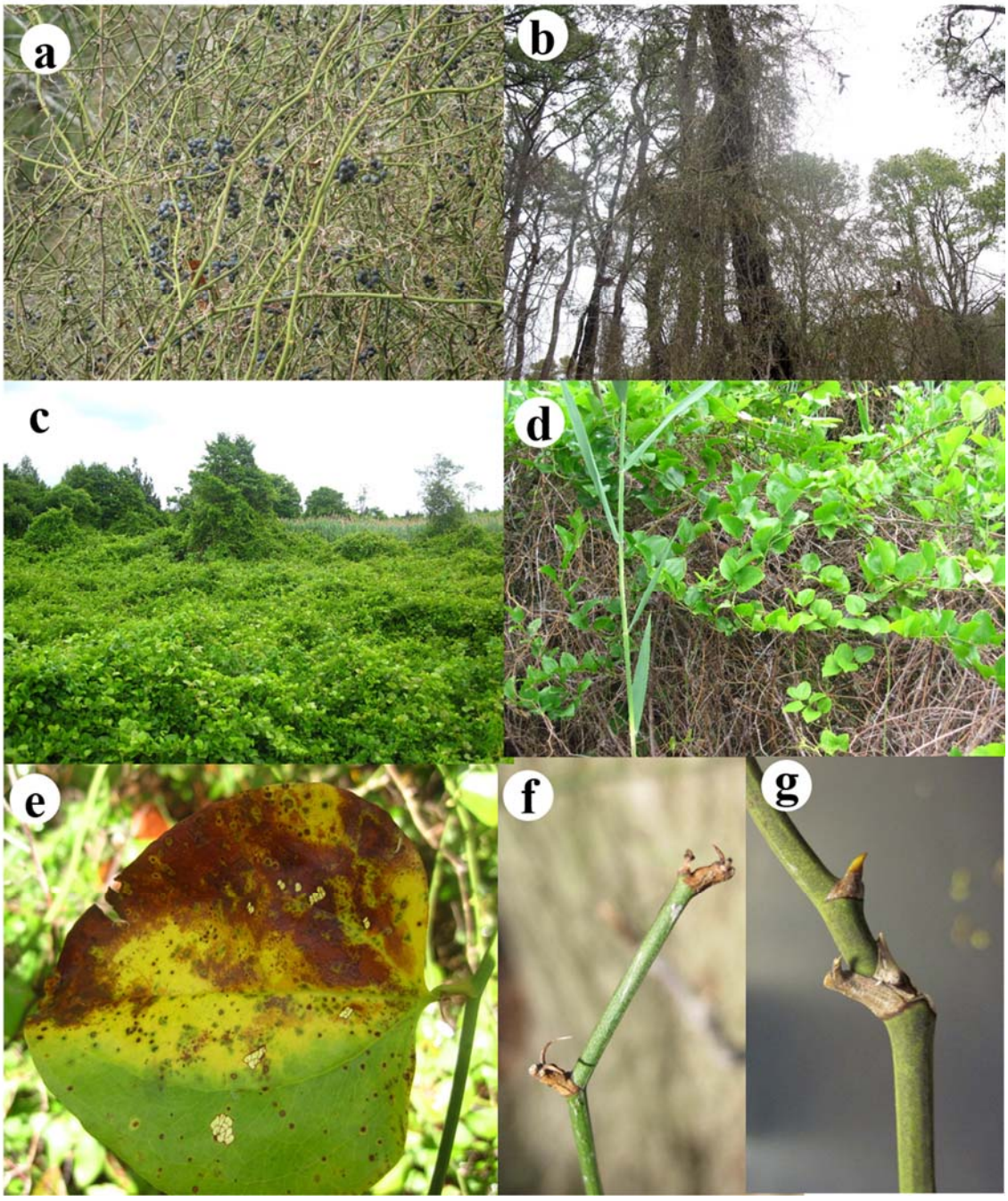

Fig. II-1. Growth habit and morphology of *S. rotundifolia*

(a) Stems with berries. **(b)** Climbing habit. **(c)** Dense expansive stand of greenbrier in open sun (no canopy) at Sandy Hook, Gateway National Recreation Area, (site SH3, Chapter 4). **(d)** Smilax living vines on top of dense mass of standing dead stems. **(e)** Typical senescence pattern in greenbrier leaf, grading from brown to yellow to green living tissue with signs of microbial attack throughout**. (f)** Greenbrier stem in the field in winter, showing remnants of tendrils and dead previous years petioles. **(g)** A common pattern in vines, where a primary stem axis has died back, and growth is taken over by a new shoot, with a hardly noticeable transition.

Animal associations

S. rotundifolia, and greenbriers in general, are an integral part of Eastern U.S. forest ecosystems. Berries provide food for birds including ruffed grouse, wild turkey, sharp-tailed grouse, prairie chickens, and ring-necked pheasant, and at least 38 species of non-game birds, and the berries persist as a resource in winter when food is scarce (Smith 1974). Stems and leaves of greenbriers are considered one of the most important deer browse plants in the South (both stems and leaves), and the tender early growth of stems is consumed by rabbits (Goodrum 1977). A study in North Carolina showed that various parts of *Smilax* species *(*berries, stems, and leaves) were the most important natural food in the diet of black bears in 5 months of the year (Landers et al. 1979). The spiny tangled growth also probably provides good cover for small mammals (Smith 1974).

Considering the importance of greenbrier as an abundant and reliable resource to larger animals, it is reasonable to expect that many insects also would make use of the plant, though there is a paucity of literature on the topic. Greenbrier plants in New Jersey did not appear to host many insects at most times of year. However, large numbers of insects were visible at certain times. I did not systematically study the insect associations, but they are recorded here, as they may relate to the fungal community from the perspective of facilitating fungal growth, fungal dispersal, or competition over food resources. The insects I commonly observed were:

(1) Dipteran burrowing larvae (observed in March)**:** Many grey elongated patches on stems could be observed, from 1 to a few cm, particularly at a site on Rutgers campus that I visited regularly. I collected samples of these in late March of 2011 and

incubated them in a moist chamber to study the fungal growth. I was surprised to find live insect larvae moving within a cavity below the epidermal layer in what is normally solid wood. A state entomological expert identified these as dipteran larvae based on their mouthparts—further identification would require rearing to adult. The larvae ate internal tissues but dead grey surface layers were left to be exploited by fungi, and pycnidia appeared on these dead surface layers in wet chambers.

(2) Ants and Nectaries (May–June): In the early period of rapid cane elongation and leaf opening in spring, diverse species of ants (widely different sizes, colors, behaviors) were very common on stems at some sites. The ants were drawn to extrafloral nectaries from the base of the petioles, on the abaxial side, below the tendrils. In a dissecting microscope, I observed ants repeatedly remove drops of nectar from these nectaries; the drop of nectar quickly replenishes to about the same size after removal. These nectaries only appear to be active during the period of rapid growth when new tissue is very tender, after which no further conspicuous ant activity is observed. As is typical of extrafloral nectaries, the ants may serve as defense from herbivorous insects before the highly palatable young growth toughens. Their constant movement across plants in spring may also facilitate dispersal of fungal propagules.

(3) Lepidopteran larvae (late August–September): Huge infestations of caterpillars, which I identified as *Phosphila turbulenta* moth larvae, consumed leaves of the plant in autumn at certain sites, particularly in the New Jersey Pine Barrens, and at Sandy Hook. Large *P. turbulenta* caterpillars also swarmed over the stems after all the leaves were depleted, though it is unclear if they are able to get further nutrition from the

stems. The activities of these caterpillars could influence fungal dispersal by spore attachment to the insects, or even movement through their digestive track and later deposition on stems. They also may compete with saprophytic fungi for weak and sometimes senescing leaf tissue that is still alive and attached to the plant in the autumn.

(4) Spider-mites: Spider-mites occasional infested individual leaves, causing the leaves to roll up, but these were scattered and rare. I never observed large infestations.

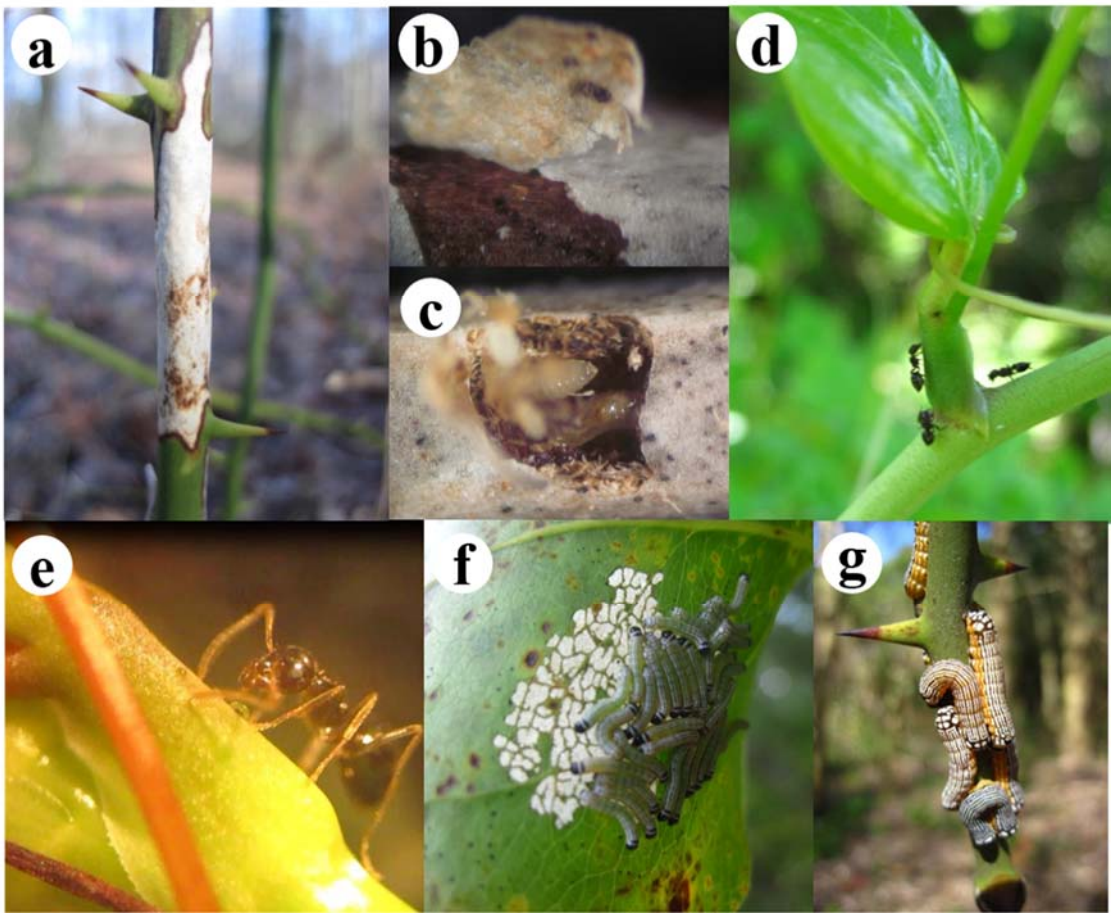

Fig. II-2. Greenbrier associations with insects

(a) Greenbrier stem in March, with discolored area indicating dipteran burrowing insects. **(b)** Outer epidermal layer easily peels off where dipteran larvae have infested stem. **(c)** Cutting into inner layers reveals hollow inner stem with dipteran larvae. Healthy stems are normally hard solid wood. Fungal structures visible as black spots, probably pycnidia, can be seen in the outer dead epidermal layer in this and the previous image. **(d)** Ants gathering around extrafloral nectary on petiole of succulent young greenbrier shoot at Sandy Hook in early June **(e)** An ant about to consume a drop of (*Continued*)

fluid from extrafloral nectary on a petiole; this shoot was cut from the field with ants still clinging to it, and sealed with parafilm within a petri dish for observation. The photograph is through a dissecting microscope. **(f)** *Phosphila turbulenta* larvae consuming greenbrier leaf tissue in the NJ Pine Barrens in September. **(g)** Larger *P. turbulenta* larvae photographed on leafless stems of greenbrier in autumn at Sandy Hook, Gateway National Recreation Area.

Mycoflora

I noticed that the plant had a resident mycoflora on living stems from observing the dark hyphal growth on many stems of the plant in winter. Lifts using clear nail varnish showed that rich hyphal growth covered some areas of the stem surface (Fig. 3). The first investigation of this growth showed that by first soaking stems, the hyphae could be lifted off the stem with a scalpel or needle and spread over solid growth media in Petri dishes. A few preliminary samples using this technique showed that *Pestalotiopsis* spp. and the related *Monochaetia dimorphospora* arose from the hyphae. Because of the obvious surface growth, it seemed that *Smilax* stems would make an excellent study subject for comparison of endophytic to epiphytic fungal communities.

S. rotundifolia vines obviously are an important resource to fungi, and the plant has been sampled by mycologists many times, but has not been studied systematically as a fungal ecosystem. The plant is interesting from a mycological perspective for several reasons. Some phyllosphere fungi, as is clear in certain parasites (Allen et al. 2004), may be coevolved with their hosts, being adapted to the defenses, and the general physical and chemical environment of the species or genus. The genus *Smilax*, having the combination of woody habit and monocot lineage is unusual among the other woody plants in the northeastern U.S. region, and so may contain a more unique group of fungi compared to the other woody dicots and gymnosperms in the region. Secondly, while trees have often been studied in endophytology, other forest layers have been relatively neglected. As

Smilax spp. make up a large portion of the understory, and be important to the ecosystem, the genus is a good candidate for study.

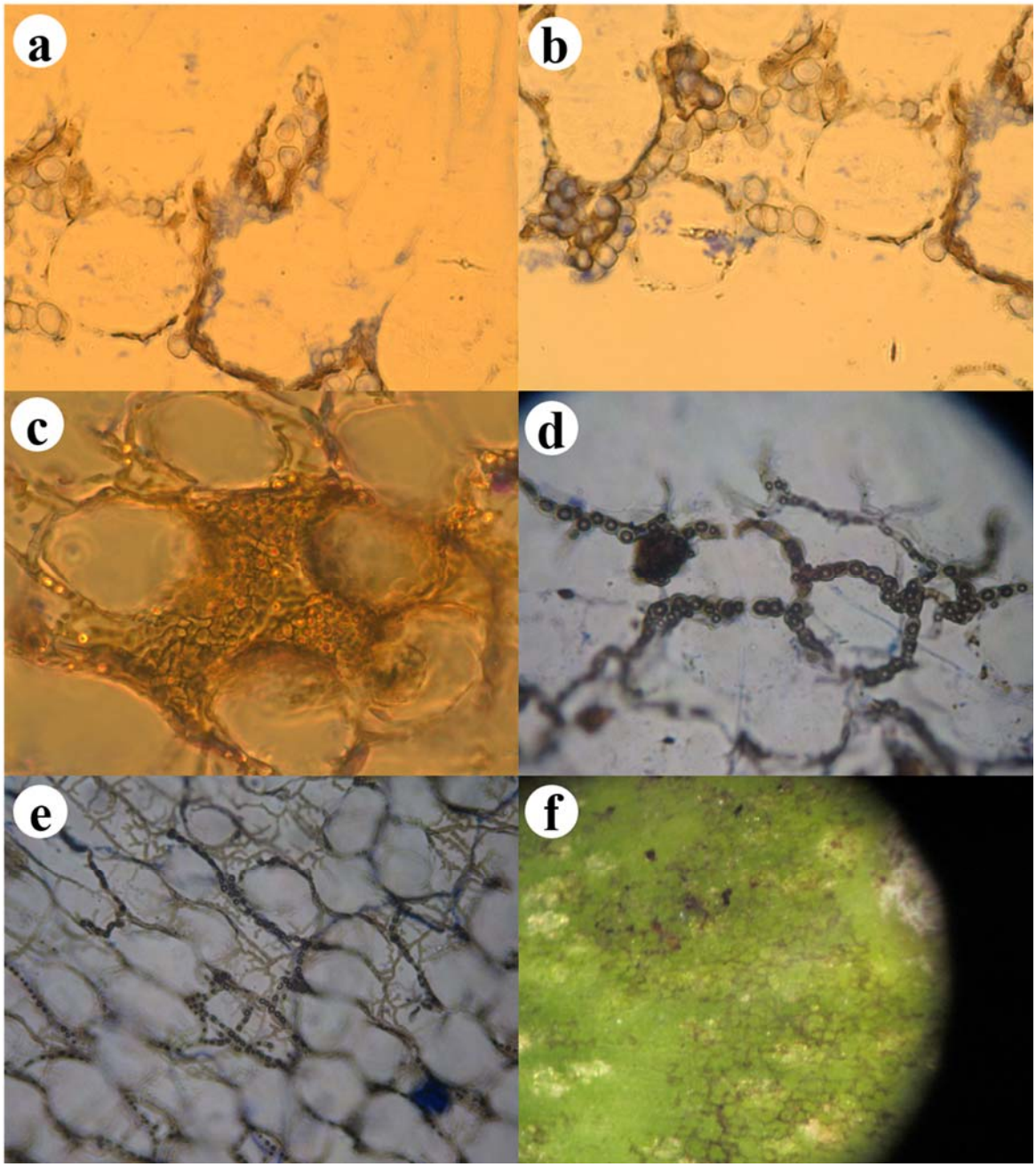

Fig. II-3. Fungal growth on greenbrier stem surfaces

The round protrusions around which the hyphae wind are epidermal cells, giving a sense of the scale. **(a-b)** Thin sections fixed in resin and stained with aniline blue. The cut was made at a slight angle to the surface; photographed under compound microscope **(c-e):** acrylic lifts, no stain added, photographed under compound microscope. **(f)** Intact greenbrier stem soaked in water so that hyphal network is more visible, no stain added, photographed under dissecting microscope.

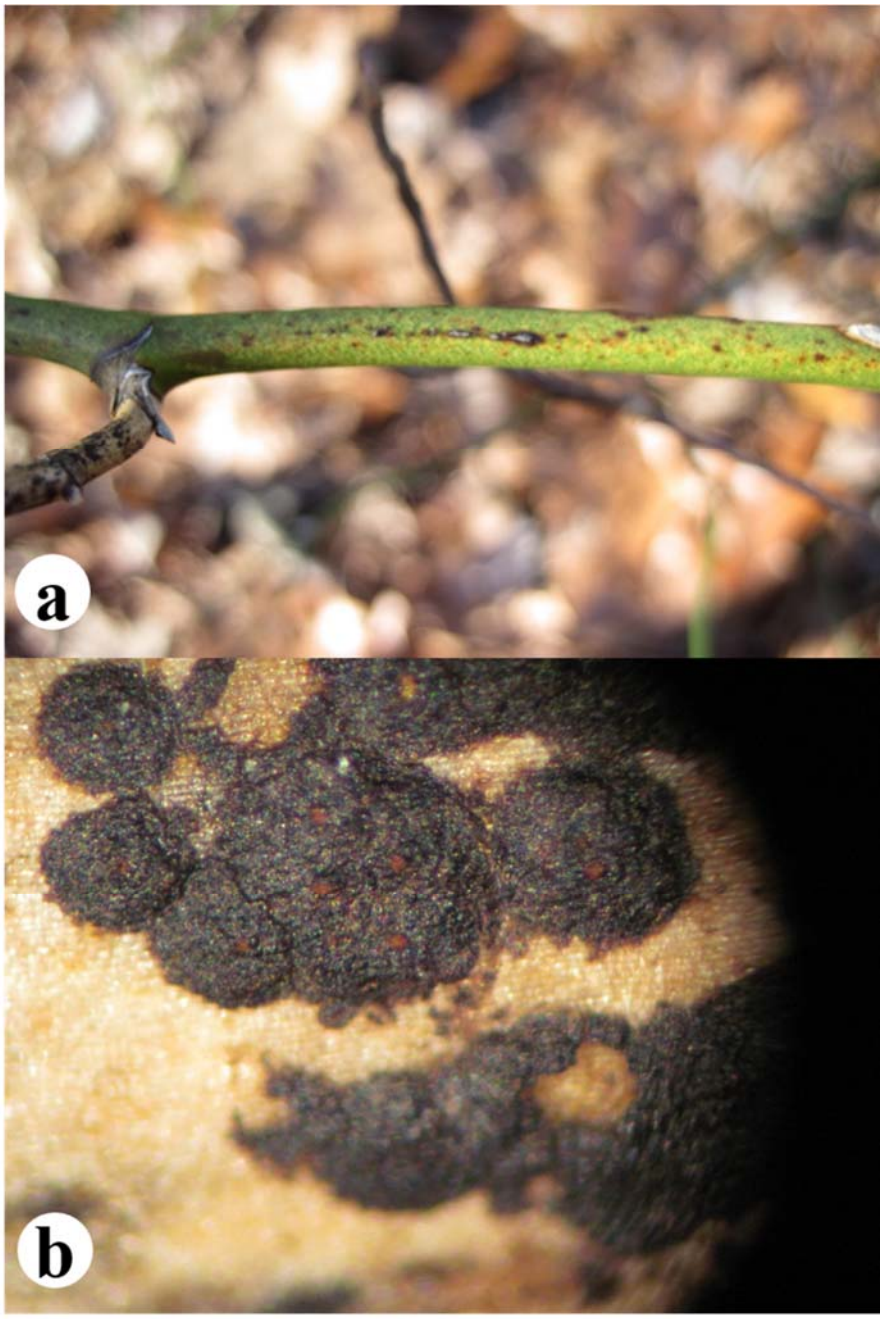

Fig. II-4. *Muyocopron smilacis* infection visible on *S. rotundifolia* stems

(a) Diseased stem showing red spots characteristic of the disease per Luttrell (1944), and dead shoot to the left side, with characteristic black growth. **(b)** *M. smilacis* sexual reproductive structures on dead *S. rotundifolia* stem photographed through dissecting microscope (asci and ascospores, not shown, were also observed using a compound microscope).

Chapter III. Fungal community of greenbrier stems in winter: niche-driven patterns of colonization and spatially correlated structure at the local scale[1]

Abstract

Endophytic and epiphytic fungal assemblages from stems of *S. rotundifolia,* a shrubby vine, were studied in order to (1) quantify differences and the degree of overlap between fungal communities of both micro-habitats, and (2) examine whether fungal assemblages are spatially correlated at the local scale (tens to hundreds of meters), in order to understand if dispersal limitation may play a role in structuring these communities. Sampling was conducted over 160 m of growth along a forest edge. The communities of the plant surface vs. interior showed low overlap (Bray-Curtis Similarity=0.22), with most species that were common in one habitat appearing rarely if at all in the other, and only *Aureobasidium pullulans* showing high frequency in both. Epiphytic assemblages proved to be spatially correlated along the 160 m length of the transect, and even more so when adjacent pairs of stems were considered as the unit of comparison rather than individual stems. Endophytic assemblages showed no significant spatial correlation along the transect. Unexpectedly, three species of endophytes showed a pattern in which abundance of colonies peaked at different heights on the stems. *Colletotrichum boninense* showed peak abundance at 3 cm from the stem base. *Phomopsis* sp. 1 peaked at 33 cm from the stem base. Endophytic isolates of *A. pullulans* peaked at 63 cm from the stem tip. It was also found that positive correlative relationships were detected between endophyte-endophyte, and epiphyte-epiphyte pairs. Cross-group interactions seemed to center around crossover species that were not entirely constrained within the surface or interior.

1. Introduction

Endophytes of aerial parts of woody plants have been studied extensively for the past several decades after it was first demonstrated that multiple taxonomically dissimilar species of fungi regularly infect the living needles of coniferous trees without causing symptoms of disease (Bernstein and Carroll 1977; Carroll et al. 1977; Petrini 1991, 1996). It has become widely accepted that almost all land plants in natural settings are inhabited by endophytic fungi (Petrini 1991, 1996). Evidence to date suggests that with the exception

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of the clavicipitaceous endophytes of grasses, these aerial endophytes are horizontally transmitted (Arnold and Herre 2003) and infections are highly localized rather than systemic (Stone et al. 2004; Boyle et al. 2001). A general pattern of abundance is typically observed in which one to several fungal species are very dominant followed by a long list of increasingly rare species, with the identity of the fungi characteristic to the plant species sampled (Petrini 1991, 1996).

A variety of factors have been studied for their potential influence on infection frequencies and composition of endophytic communities, including the effect of latitude (Arnold and Lutzoni 2007), temperature and rainfall (Zimmerman and Vitousek 2012), host genetics vs. environment (Elamo et al. 1999; Balint et al. 2013), host species (Sun et al. 2012; Wearn et al. 2012; Persoh 2013; Davey et al. 2012), organ/tissue type (Wang and Guo 2007; Wearn et al. 2012; Mishra et al. 2012), seasonal change (Wearn et al. 2012; Mishra et al. 2012; Davey et al. 2012; Jumpponen and Jones 2010), and geographic locality (Mishra et al. 2012; Persoh 2013; Davey et al. 2012). However, one aspect of endophyte ecology that has been almost completely unstudied is the efficiency of movement, through dispersal of propagules, of endophytic species at the local scale. "Local" is defined here to mean at the scale of a stand of plants, such as a grove of conspecific trees or a thicket of woody shrubs spanning tens of meters to several hundred meters in any direction. This is to be distinguished from dispersal at the landscape scale between patches of plants a few kilometers to hundreds of kilometers apart. Because endophytic infections are often restricted to one or a few cells (Stone et al. 2004), the presence of several dominant endophytes within a site cannot be expected to ensure dispersal of these species to all receptive plant tissues, and the amount of tissue infected

by each species may vary considerably from plant to plant. If dispersal of micro-fungi is slow and limited throughout a stand of plants, the dynamics of fungal interaction would obviously differ from those of a system in which dispersal of several dominant species is nearly perfect to all available plant tissue.

Interactions between phyllosphere species have previously been shown to be influenced by propagule density and interspecies antagonism in vitro (Nix-Stohr et al. 2008). Local dispersal limitations could be important in relation to how often endophytic species interact, how often they compete, and in determining the final community structure that is observed. The importance of limited versus universal dispersal at the local scale has been demonstrated by ecological models under assumptions of both neutral and niche-based theory (Chave et al. 2002; Adler et al. 2007), and even in experimental microcosms using bacteria (Kerr et al. 2002), in which it has been shown to result in greater community diversity and the coexistence of more potential competitors. In one ecological model of coexistence that easily translates to endophytic systems, the competitive ability of one species to dominate a small patch and displace competitors may be balanced by higher fecundity and dispersive abilities of other species (Amarasekare 2003).

Efficiency of dispersal can be assessed indirectly by intensive sampling along a transect of growth of the same plant species that is lacking in any obvious environmental gradient that might be expected to affect microbial fitness. Perfectly even dispersal across a site should be reflected in a lack of spatial correlation between assemblages of species gathered at different points. If assemblages from various distances are spatially

correlated, then dispersal limitation is the likely explanation. It should be noted, however, that in this approach recruitment is what is actually being measured as a proxy for dispersal, as sampling reflects not only the ability of propagules to reach viable plant tissue throughout the site, but to colonize and survive until the time that a sample is taken.

A good plant candidate for the study of micro-fungal spatial structure was found in 'greenbrier' *(Smilax rotundifolia),* an abundant shrubby vine native to the northeastern United States. Greenbrier often forms large shrubby thickets, and is well known for its painful thorns and sometimes impassibly dense growth. Since growth of the plant is often expansive, spreading over tens to hundreds of meters, sampling of stems is possible at regular intervals within a stand of plants to study spatial aspects.

The *Smilax* genus is in family Smilacaceae (Liliales), and contains over 200 species, including lianas, shrubs, and herbs in the genera *Smilax* (~200 sp.) and *Heterosmilax* (~12 sp.) (Qi et al. 2013). The only investigation to date of endophytic colonization in this family was a culture independent, clone library-based study on stems of *Heterosmilax japonica* done at the South China Institute of Botany, PR China (Gao et al. 2005).

As a monocot, *S. rotundifolia* lacks secondary growth of bark, and its stem, while tough and providing some vertical structure, also retains a green photosynthetic function. Epiphytic fungal populations are often visible as sooty growth contrasting against the green of the stems. Preliminary investigations conducted by our lab had shown stems of the plant to be rich in a variety of both endophytic and epiphytic fungal colonies

suggesting that it would not only make a good model to study spatial structure, but also might contain a relatively large component of unexplored biodiversity.

Although there is a history of research comparing endophytic versus epiphytic populations of fungi (Kendrick and Burges 1962; Macauley and Thrower 1966; Ruscoe 1971; Watson et al. 1974; Wildman and Parkinson 1979; Cabral 1985; Osono 2002; Osono et al. 2004; Osono and Takeda 1999; Legault et al. 1989a, b; Santamaria and Bayman 2005; Kharwar et al. 2010; Osono and Mori 2004; Osono 2008), these groups have never been compared from the spatial perspective.

The objectives of this study were defined as follows:

1. Describe endophytic and epiphytic fungal assemblages of greenbrier stems and determine if they are distinctly different groups.
2. Explore efficiency of local dispersal; test for correlation between spatial distance and dissimilarity of microfungal assemblages sampled at ranges from <1 m to 160 m apart.
3. Test for correlative evidence suggesting possible species interactions, particularly whether endophytes and epiphytes might interact with each other.
4. Test whether the position from which samples are taken on the stem (distance from base or tip) influences the colony abundance of dominant species.

2. Materials and methods

2.1 Sampling design, collection, and processing

The study was focused on a single transect of 160 m of greenbrier growth using a nested sampling design so that multiple comparisons could be made between spatial distance and dissimilarity of fungal assemblages at different distances ranging from < 1 m to 160 m apart. Stem tissues were sampled but not leaves, partly because the time

frame for sampling was in late winter/early spring previous to emergence of new leaf growth.

The study site was located at the eastern edge of the George H. Cook Campus of Rutgers University (New Brunswick, NJ, USA), where about 14 ha of forested land are situated between the campus and the neighboring property. In an area of forest bordering on a field (40°28'28.0"N, 74°26'14.0"W) *S. rotundifolia* was found nearly continuously for at least 160 m just within the forest edge (with some areas of gaps and sparse growth). A 160 m transect was measured along this growth parallel to the forest edge, and marked at meters 0, 10, 50, 60, 100, 110, 150, and 160. At each of these points, 2 stems from within 1 m of the marker that were not visibly arising from the same rhizome were selected for sampling. The selected stems (16 total) were removed in pairs every 3-4 days over the time period of March 10, 2010 to April 6, 2010. Pairs of stems were removed in a mixed order from meter markers along the transect in the order of 0, 10, 160, 150, 50, 60, 110, and 100 m.

Stems were cut at ground level in the field, brought to the lab, and stripped of branching to one central axis. A total of 10 segments were then marked to be cut from each stem as follows: starting 3 cm from the base, five 1-cm segments were marked at 15 cm intervals, then starting at 3 cm from the tip, another five 1-cm segments were marked at 15 cm intervals. Samples were originally cut at 4-cm size, and notched with a razor to convey which stem position they had been cut from before washing. Samples from the same plant were washed in a beaker (with metal mesh cover) under flowing tap water for 30 min. After the initial wash, these were blotted dry on sterile paper towels, then

trimmed to exactly 1-cm. From this point forward, stem position was tracked with labeled wash containers, towels, or Petri dishes so that each sample could be traced to stem and stem position.

For epiphytic isolations, segments were individually washed with 1 min of manual shaking in 3 changes of sterile distilled water in a pre-sterilized 110-ml jar, then air dried on autoclaved paper towels in a sterile hood for 3 h. Washing was to remove transient spores. Drying was to reduce bacterial growth (Osono and Takeda 1999). A piece of tape (3M #600) was stretched over a U shaped glass rod, then placed sticky side up on a flame-sterilized (i.e. 95% ethanol plus flame) glass slide. Using flame-sterilized tweezers, 1-cm stem samples were rolled back and forth two times over a 2-cm distance (marked with lines beneath the glass slide), and pressed down repeatedly while rolling. Tape was then cut so that all edges were removed and only the 2 x 1 cm mark from the stem remained. The tape was cut into 8 pieces using flame-sterilized tools, which were placed sticky side down on a standard size plate of Malt Extract Agar (MEA) (Difco). Plates were sealed with Parafilm® and placed in plastic containers at lab ambient light/temperature to await colony isolation. In some cases, after this epiphytic isolation process, stems were stored overnight in sterile Petri dishes at 4°C before commencing endophytic isolations.

For endophytic isolations, the same segments were then placed in a 110-ml jar and manually shaken for 1 min in 70% ethanol, then 3 min in 3.1% NaOCl (50% Clorox), then 4 consecutive washes for 1 min each in sterile distilled water. After rinsing, the segments were left in the hood to dry for 3 h on autoclaved paper towels. Samples were

subsequently cut using flame-sterilized razors into 12 thin discs that were placed together on an MEA plate. However, some thick stem segments were too hard to be cut cleanly into 12 discs, so these were first halved vertically and horizontally, then sliced so that 12 half discs were produced and plated. A total of 1,920 stem slices and 1,280 thin cuts of tape were plated on 320 plates (160 endophytic samples + 160 epiphytic samples), representing 160 1-cm segments, and 16 plants.

To test that the tape used for epiphytic isolations was clean, samples of tape fresh from the roll were cut according to the isolation procedure and plated on MEA in three Petri dishes, then observed over the course of 3 weeks. This resulted in no growth of any organisms, which was taken to indicate that the interior of tape rolls was reasonably sterile and the tape was not contributing to the species recorded. Additionally, to validate sterilization efficacy, three stems were surface disinfected, pressed into MEA and rolled across it, then left for 10 min before removal. These produced no colonies, indicating that surface disinfection was probably sufficient to kill all surface organisms most of the time.

After epiphytic and endophytic samples had been plated for 4 days, isolations were made. For endophytic samples, tissue segments that had no growth were transferred to fresh MEA plates to avoid overgrowth. For epiphytic samples, growth was so prevalent and fast that this was not attempted. Plates were examined again at 8 days, 2 weeks, and then intermittently for additional growth over a 6-month period. Each morphotype was isolated one time from a plate; thus if a plate had 10 very similar colonies in appearance and growth rate, then only 1 was isolated, while the others were recorded.

Morphotypes were designated by first matching macroscopically similar colonies in color, texture, and growth rate, then by comparing conidial morphology if available. For each sporulating colony, conidia were examined microscopically using a Zeiss Axioskop Compound Microscope, photographed, and photographs later compared. Macroscopic colony morphology on MEA was also photographed for each isolate (top and bottom of plate). Final morphotypes were designated based on a combination of macroscopic and conidial morphology. Separate morphotypes were designated only when consistent differences were seen in either microscopic or macroscopic features or both. To encourage sporulation, non-sporulating colonies were transferred to Oatmeal Agar (2.0 % Quaker brand oatmeal boiled for 15 min and strained through cheesecloth, 2.0 % Agar), V8 agar (20 % V8 vegetable juice by volume, 2.0 % Agar, 0.3 % $CaCO3$), and moist chambers with autoclaved greenbrier stems (i.e. Petri dishes with sterile towel paper moistened in sterile water, sealed with Parafilm®).

Some idiosyncrasies of working with overwintered vines were dealt with as follows: Since variables other than spatial distance were purposely avoided, roughly similar length vines were selected for sampling. Average vine length was 196 cm, with a range of 145-381 cm. None of the over-wintered vines were unblemished, and plants with wounds from insects, disease, or the loss of multiple side shoots were not excluded from sampling as these were typical and unavoidable. When cutting the 1-cm segments for sampling, adjustments of about 0.5 cm or less were made to avoid areas that showed major wounds, possible insect damage or disease symptoms. But small light brown marks (possibly wound scars) on the stem were common, especially when viewed under a dissecting microscope, and could not be avoided. Finally, in designating the central stem

axis for sampling, it was found that the original stem axis sometimes stopped short of the length required for the sample procedure, but the axis of thickest growth was taken over by what had been a side shoot. Often the transition was so smooth that this was not noticed until the stems were brought back to the lab and closely examined; these transitions were recorded during sampling.

2.2 Scoring of samples and smoothed sampling curves

Presence or absence of colonies was recorded for each subdivided slice of tissue (12 per plate) or each cut of tape (8 per plate), and this dataset is referred to as the "colony count data." This data was used for testing for interspecies correlations (objective 3) and abundance of colonies at different stem positions (objective 4). For the purposes of generating smoothed sampling curves, comparing diversity, and calculating metrics of overlap between endophytic and epiphytic assemblages, data was reduced to a single presence/absence record of each morphotype per 1-cm segment, referred to as the "presence/absence dataset." This was done to overcome inconsistencies in the sampling methods, as fewer segments of tape were represented per segment compared to the number of sterilized tissue fragments, and because rolling stems across tape may have picked up the same mycelium more than once.

The program EstimateS v9.1.0 (Colwell 2013) was used to create smoothed sampling curves for species richness, Shannon exponential (e^H), and for estimators of species richness (Chao 2, etc.). Data was analyzed for endophytes, epiphytes, and a combined dataset. The data was treated as multiple sets of replicated sampling units (as opposed to individual-based sampling); each plant, containing 10 x 1-cm segments and

thus 10 possible occurrences per species, represented a sampling unit. Since the same stem segments were used for both surface and interior sampling, presence on the surface and interior of the same segment was only counted once in the combined dataset.

Smoothed sampling curves were randomized using 500 bootstrap replicates without replacement in estimates of species richness, using the variable 'S(est),' in EstimateS and confidence intervals for the same (Colwell et al. 2004); bootstrapping was done *with replacement* for estimates of e^H so that standard deviations could be generated without converging to zero in the last few samples (options for 95 % confidence intervals are not available in EstimateS for e^H). As species richness curves did not plateau, estimators of species richness were assessed as well (ACE, ICE, Chao 1 & 2, Jackknife 1 & 2, Bootstrap, and Michaelis-Menten).

2.3 Statistics, ordination, diversity & similarity measures

The Bray-Curtis index (Bray and Curtis 1957) and Sorensen quotient of similarity (Sorensen 1948) between endophytic and epiphytic assemblages were generated using PAST v3.01 (Hammer et al. 2001), to generate matrices of similarity indices, under the type: "Bray-Curtis," for Bray-Curtis, and "Dice" for the Sorensen QS. The Shannon index (H') was calculated in R v3.0.1, using package vegan 2.0-10, function 'diversity {vegan}', using the default natural log. The Shannon index was converted to Shannon exponential (e^H) before calculating the ratio of endophytic to epiphytic diversity.

To test for whether there was a significant difference between surface and interior assemblages, the non-parametric test Analysis of Similarities, or ANOSIM (Clarke 1993) was used in the program PAST. Differences between groups were visualized using non-

metric multidimensional scaling (NMDS) in 2 dimensions, also as implemented in PAST (which uses the method of Taguchi and Oono 2005). The Bray-Curtis similarity was used as the similarity measure in both ANOSIM and NMDS.

Rank abundance plots were generated in R package BiodiversityR. Proportional abundance was plotted against rank. Proportional abundance in the program is calculated as (counts of each species + total counts of all species) x 100.

The Mantel test (1967), also implemented in PAST, was used to assess whether Bray-Curtis distance (1 - Bray-Curtis similarity) between fungal communities was correlated to spatial distances over a 160 m length of forest edge. The test was applied both to the 16-stem dataset, and also to a dataset in which stem pairs from the same meter marker (0,10, 50,60, 100, 110, 150, 160 m) were combined to accentuate location over inter-stem variation. The test was applied separately to endophytic vs. epiphytic datasets. All corresponding points of geometric distance and Bray-Curtis dissimilarity were also plotted, and a linear regression line generated in Excel.

Correlations between species were tested in PAST with the colony count dataset, using the correlation statistic Kendall's tau. This is a non-parametric test of association, appropriate because data were non-normally distributed. To restrict the number of comparisons, only species with over 28% frequency were tested (10 species total). P-values were Bonferroni corrected for multiple comparisons. A chi-square test of contingency was conducted on frequencies of species occurrences from each stem in order to determine if 1-cm segments taken from the same stem could be considered independent samples.

Significant differences in species abundance counts at each stem position were tested for using R package Agricolae v1.1- 8 (using colony count data). Using the function kruskal{agricolae}, the Kruskal-Wallis test was used to test for overall significance and pairwise significance of stem position on the abundance of colonies for the five most abundant endophytes. The p-value alpha was set to 0.05, and the Holmes correction was used for multiple comparisons. Results were visualized using bar.err{agricolae}.

2.4 Molecular identification

For frequently occurring morphotypes that were sterile or were sporulating but difficult to identify to genus, molecular identification was attempted using the ITS region and a reference database. To produce biomass of pure mycelium for DNA extraction, isolates were cut to small pieces and seeded to a 125-ml flask with Potato Dextrose Broth (Difco), then put on a shaker for 1-3 weeks. Some mycelium was removed, squeezed dry on a sterile paper towel, and DNA was extracted from approximately 25 mg of mycelium using the Mo-Bio Plant DNA extraction kit following the manufacturer's protocol.

For PCR amplification, the primers ITS1 and ITS4 (White et al. 1990) were used with an initial denaturing step of 5 min at 95°C, followed by 37 cycles of 30 s at 95°C, 57 s at 57°C, and 57 s at 72°C and a final extension step of 7 min at 57°C. In several cases the forward primer ITS1 and reverse primer LR5 (Vilgalys and Hester 1990) were used, with amplification parameters of 2 min at 95°C initial denaturing, followed by 37 cycles of 30 s at 95°C, 57 s at 57°C, and 90 s at 72°C, and a final extension step of 10 min at 72°C. Additionally, for one morphotype a portion of the Large Subunit rRNA coding

region was sequenced using the primers LROR and LR5 (Moncalvo et al. 1995), with initial denaturing step of 4 min at 94°C, followed by 37 cycles of 1 min at 94°C, 1 min at 52°C, and 1 min at 72°C, and a final extension step of 10 min at 72°C.

QIAquick PCR purification kit (Qiagen) was used for purification of PCR product, which was sent to Genewiz, Inc. (Piscataway, NJ) for Sanger DNA sequencing using the same forward and reverse primers used for amplification.

Forward and reverse sequences were aligned and edited in SeqTrace 0.8 (Copyright 2012, Brian J. Stucky). Longer DNA sequences derived using the ITS1 and LR5 primer set were subsequently trimmed to show only the ITS1-5.8S-ITS2 region by alignment of the LROR primer (a forward primer of the Long Subunit region) and removal of all nucleotides from the LROR primer continuing in the 5' to 3' direction. This sequence trimming was performed using the program ApE (A plasmid Editor v2.0.45, Copyright 2003-2009 M. Wayne Davis).

For identification of genera, the UNITE database (Koljalg et al. 2013) general FASTA release version 6 ("sh_general_release_09.02.2014"), was downloaded from http://unite.ut.ee/repository.php. This was set up as a local database using the program BioEdit v7.2.5 (copyright 19972013, Tom Hall), and used for local Blastn searches (Altschul et al. 1997) under default search parameters of BioEdit. The database downloaded contains 17,782 'repS' and 3,056 'refS' sequences. Both sets of sequences are originally from Genbank. The repS sequences represent species hypotheses generated by a computer algorithm and not yet reviewed by experts, while refS sequences have

been reviewed by various experts for accuracy. In order to only display the most meaningful matches, the closest match to a refS sequence was recorded, while repS sequences were disregarded. Sequences from this study were also entered in the local database, and examined for percent similarity to determine if any were very similar or perfect matches.

3. Results

3.1 Objective 1

Endophytic samples (1-cm segments, surface sterilized) yielded 1,328 recorded colonies, 92% colonization (148 of 160 samples), median 8 colonies, 3 species (range: 0-8) per sample. Surface samples (1-cm segments, plated tape lift) yielded 1,162 recorded colonies, 98% colonization (156 of 160 samples), median 7 colonies, 5 species (range: 0-16) per sample. The presence/absence dataset contained 451 presence records for endophytic fungi, and 711 presence records for surface fungi.

The smoothed sampling curve for interior, surface, and combined fungal species did not plateau, indicating that the site was not exhaustively sampled for culturable fungi (Fig. 1). Multiple estimators of species richness were assessed, but only the Chao 2 (Chao 1984, 1987) appeared to be leveling off and stabilizing within the number of sample units observed, so only this estimator is shown (Fig. 1). The observed number of morphospecies for interior, surface, and combined datasets were 51, 65, and 90 respectively. The Chao 2-based estimates for the same were 96, 98, and 152 spp. While the trajectory of species richness curves and similar Chao 2 estimators might leave some room for doubt as to greater species richness, the curve for Shannon exponential was beginning to plateau and was clearly higher in the epiphytic assemblage.

The NMDS ordination (Fig. 2) of surface and endophytic assemblages from each stem showed a broad split between the two groups, and the ANOSIM test between these groups gave a very highly significant p-value and very high R value (Table 1). Most species packed neatly into the 95% confidence intervals shown in the NMDS for endophytes or epiphytes. The one endophytic assemblage that fell far outside of the ellipse, plant A at 160 m, was almost completely lacking in counts of the three most dominant endophytes. It also contained the highest counts of *Colletotrichum acutatum,* moderate levels of *Phomopsis* sp. 2, and the only two occurrences of *Pestalotiopsis* sp. 1 in endophytic samples. The species lists for the two microhabitats (Table 2) also demonstrate that most of the highly abundant species show high frequency in either surface or interior but not both, with the exception of *Aureobasidium pullulans* which was found in 50% of surface samples, and 31% of interior samples.

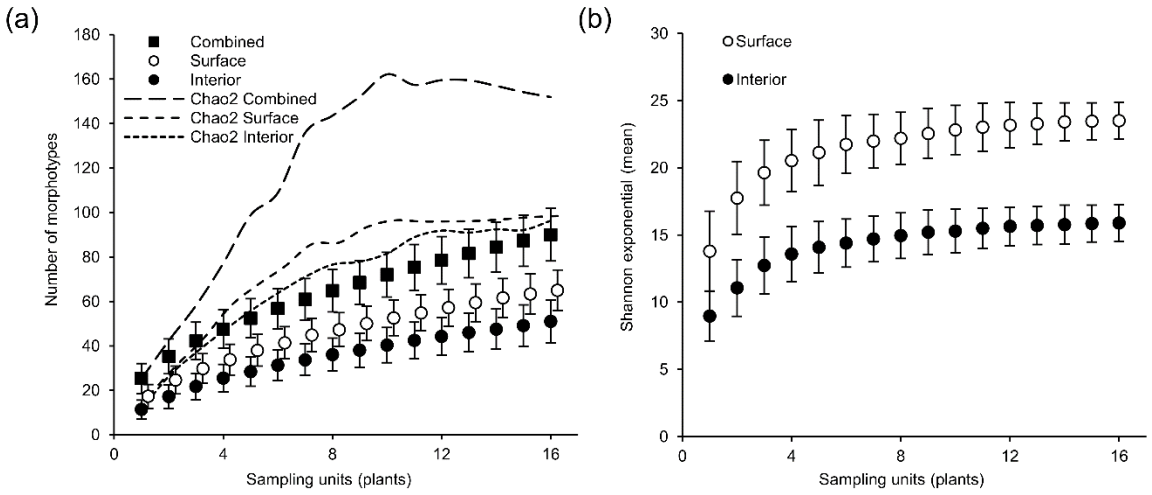

Fig. III-1. Smoothed sampling curves, fungi of greenbrier stems in winter

(**a**) Mean species richness in surface, interior, and combined datasets using 500 bootstrap replicates without replacement; error bars show 95% confidence interval; dotted lines show Chao 2 estimator of total species richness estimated at each level of sampling. (**b**) Comparison of mean Shannon exponential (diversity measure) in surface and interior datasets using 500 bootstrap replicates with replacement; error bars are standard deviation.

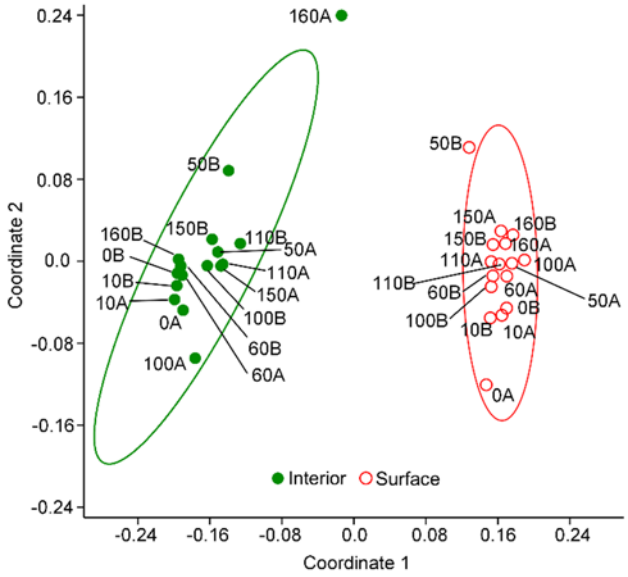

Fig. III-2. NMDS ordination of surface and interior assemblages

NMDS Ordination (stress value=0.144) in two total dimensions of surface and endophytic assemblages taken from 16 stems, with 95% confidence ellipses. Each assemblage is labelled by a number representing the position along the transect (in meters) and the letter A or B representing each of the two stems taken near each position on the transect.

Table III-1. Comparative measures of epiphytic and endophytic fungal assemblages and ANOSIM results

Unit for assemblage	Stems (N= 16)	Full dataset
Bray-Curtis similarity	0.19±0.10	0.22
Sorensen's QS	0.26±0.12	0.45
Diversity Ratio (e^H surface/ e^H interior)	1.6±0.5	1.5
ANOSIM: p-value	0.0001	-
R	0.9506	-

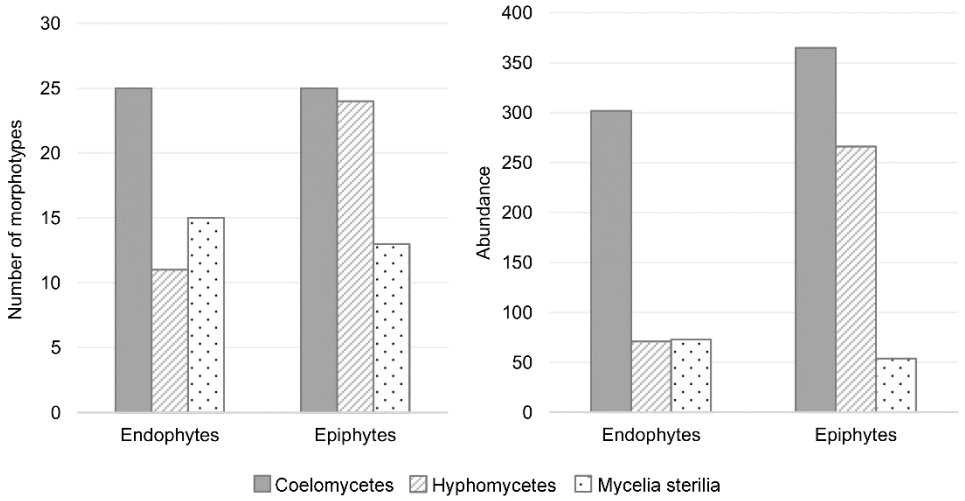

Fig. III-3. Comparison of fungal growth forms between stem surface and interior

Species richness and abundance of fungal growth forms are compared. Abundance was calculated from presence/absence dataset (i.e. presence on the 1-cm scale)

Turnover of community composition from surface to interior as estimated by Bray-Curtis dissimilarity (i.e. 1 - Bray-Curtis similarity) was approximately 80%, with a similar value whether the whole community or the average of each stem was considered (Table 1). Turnover based on Sorensen's QS (i.e. as the dissimilarity 1 - Sorensen QS) was 55% for the whole site, but was 74% ± 12 for individual stems. The diversity of the surface was calculated to be 1.5x that of the interior in terms of e^H (e^H interior=17.0, e^H surface=24.9). This ratio seems to be robust to sample size, as the average ratio over all 16 stems was similar (Table 1).

Analysis of the fungal growth forms (Fig. 3) showed that the endophytic and surface isolations both yielded more coelomycetes than other groups, with an equal 25 coelomycete species in both habitats. They differed though in that the surface samples contained proportionally far more hyphomycetes. The lowest species richness was shown for mycelia sterilia spp. in both habitats. In terms of abundance, a very similar trend was observed.

The common surface genera (common defined as >10 % frequency) were *Aureobasidium* (1 sp.), *Pestalotiopsis* (3 spp.), *Cladosporium* (1 sp.), *Coniothyrium*-like forms (5 spp.), a *Phoma*-like form (1 sp.), *Sporobolomyces* (1 sp.), and *Tripospermum* (1 sp.) and another *Phoma*-like morphotype was observed at 9 % frequency. Blast results using the UNITE database (Table 3) placed all of the four *Coniothyrium*-like morphotypes that were examined (a fifth was not sequenced) and one of the two *Phoma*-like species examined within the Pleosporales. Of the five morphotypes in Pleosporales, two were placed in family Montagnulaceae and three were placed as incertae sedis. The other *Phoma*-like sp. could not be confidently placed due to low query coverage, but its closest match was also within Pleosporales. None of the mycelia sterilia or unidentified coelomycete morphotypes that were sequenced for ITS showed 100% sequence similarity between different morphotypes. This, combined with observed colony or conidial differences, supported their being counted as separate species.

Table III-2. Mycobiota of *S. rotundifolia* caulosphere in late winter/early spring

I. Stem endophytes

			Comparison to stem surfaces	
Name	Abundance[1]	Frequency[1]	Abundance	Frequency
Phyllosticta sp. 1	76	48 %	0	0 %
Phomopsis sp. 1	62	39 %	0	0 %
Colletotrichum boninense sensu lato sp.	56	35 %	0	0 %
Aureobasidium pullulans	49	31 %	80	50 %
Mycelia sterilia A-Tan*	46	29 %	9	6 %
Phomopsis sp. 2	22	14 %	2	1 %
Colletotrichum acutatum sensu lato	15	9 %	0	0 %
Botryosphaeria sp. 1	15	9 %	4	3 %
Coniothyrium-like sp. 3**	8	5 %	22	14 %
Phyllosticta sp. 2	8	5 %	0	0 %
Phoma-like sp. 1*	8	5 %	43	27 %
Coniothyrium-like sp. 2A**	8	5 %	28	18 %

II. Stem epiphytes

			Comparison to stem interiors	
Name	Abundance	Frequency	Abundance	Frequency
Aureobasidium pullulans	80	50 %	49	31 %
Pestalotiopsis sp. 1	63	39 %	2	1 %
Pestalotiopsis sp. 2A	59	37 %	3	2 %
Cladosporium cladosporioides	59	37 %	6	4 %
Coniothyrium-like sp. 1A***	49	31 %	1	1 %
Coniothyrium-like sp. 1B***	44	28 %	1	1 %
Phoma-like sp. 1*	43	27 %	8	5 %
Pestalotiopsis sp. 2B	37	23 %	0	0 %
Trispospermum myrti	34	21 %	0	0 %
Coniothyrium-like sp. 2A**	28	18 %	8	5 %
Sporobolomyces sp. (Red Yeast sp.)†	24	15 %	0	0 %
Coniothyrium-like sp. 3**	22	14 %	8	5 %
Coniothyrium-like sp. 2B	18	11 %	3	2 %
Phoma-like sp. 2***	14	9 %	1	1 %
Mycelia sterilia C*	13	8 %	5	3 %
Mycelia sterilia D	10	6 %	1	1 %
Mycelia sterlia B	10	6 %	1	1 %
Diplodia sp.(*Botryosphaeria* sp. 2)†	9	6 %	0	0 %
Mycelia sterilia A-Tan*	9	6 %	46	29 %
Ascochyta-like sp. 1	8	5 %	0	0 %

(Only morphotypes at 5 % frequency or higher are listed. In the endophytic dataset, there were 39 additional morphotypes not shown in the table for a total of 51. In the epiphytic dataset, there were 45 additional morphotypes not shown in the table for a total of 65)

[1] 'Abundance' is simply the number of presence counts in 1-cm segments (of 160 possible); 'Frequency' is (abundance ÷ total no. of samples) × 100;

† Information from UNITE database (see Table 3) incorporated into genus shown in table above;

*UNITE: Inconclusive, **UNITE: Pleosporales, Montagnulaceae, ***UNITE: Pleosporales, incertae sedis

Table III-3. ITS BLAST matches using UNITE database for common morphotypes that were difficult to identify to genus[1]

Morphotype	Accession no.	Isolate	% Identity	Query Coverage	Highest scoring "refs" match from UNITE database	Phylum, Class, Order, Family of match
Red yeast sp.	KP122300	10B-T-2t-1	99 %	95 %	*Sporobolomyces elongatus* (AF444561)	Basidiomycota, Microbotryomycetes, Sporidiobolales, incertae sedis
Mycelia sterilia A-Tan	KP122256	50A-N-5t-2	na	na	none	Unknown
Mycelia sterilia B	KP122263	PB1-P3-N2-4	na	na	none	Unknown
Mycelia sterilia C	KP122265	RU3-P3-N1-1	95 %	42 %	*Lecythophora luteoviridis* (HE610333)	Ascomycota, Sordariomycetes, Coniochaetales, Coniochaetaceae
Phoma-like sp. 1	KP122257	60B-T-3t-3	95 %	42 %	*Stagonospora perfecta* (KF251258)	Ascomycota, Dothideomycetes, Pleosporales, Phaeosphaeriaceae
Phoma-like sp. 2	KP122266	RU2-P1-T3-3	99 %	89 %	*Phoma dimorpha* (GU237835)	Ascomycota, Dothideomycetes, Pleosporales, incertae sedis
Coniothyrium-like sp. 1A	KP122258	60A-T-1b-7	98 %	95 %	*Macroventuria anomochaeta* (GU237881)	Ascomycota, Dothideomycetes, Pleosporales, incertae sedis
Coniothyrium-like sp. 1B	KP122259	50A-T-3b-1	99 %	95 %	*Didymella urticicola* (GU237761)	Ascomycota, Dothideomycetes, Pleosporales, incertae sedis
Coniothyrium-like sp. 2A	KP122260	160A-T-1t-4	94 %	80 %	*Paraconiothyrium variabile* (EU295639)	Ascomycota, Dothideomycetes, Pleosporales, Montagnulaceae
Coniothyrium-like sp. 3	KP122261	160B-T-5t-7	95 %	91 %	*Paraconiothyrium variabile* (EU295639)	Ascomycota, Dothideomycetes, Pleosporales, Montagnulaceae
Botryosphaeria sp. 2	KP122262	60A-T-5b-7	96 %	94 %	*Diplodia malorum* (GQ923865)	Ascomycota, Dothideomycetes, Botryosphaeriales, Botryosphaeriaceae

[1] Matches with very low query coverage were not considered reliable for inference of phylogenetic placement, so for these the UNITE database search was considered inconclusive in Table 2

The common endophytes (>10% frequency) were a *Phyllosticta* sp., a variety of *Colletotrichum boninense*, two *Phomopsis* spp., *Aureobasidium pullulans*, and Mycelia sterilia A-Tan. There were also an additional *Colletotrichum* and *Phyllosticta* sp. that were less common (5-10 % frequency range). The morphotype Mycelia sterilia A-Tan was not strictly sterile, as some colonies produced apparent spermatia. These matched descriptions given by Luttrell (1944) of spermatia formed by *Muyocopron smilacis*, a parasite that, according to Luttrell, infects tips of *S. rotundifolia* canes and kills them over a number of years. Evidence of *Muyocopron smilacis* parasitism, including stem die back and fruiting bodies on brown dead stems, is evident wherever *S. rotundifolia* is found (personal observation), including the site sampled in this study.

Muyocopron was not represented by ITS sequences in Genbank (or UNITE by extension) at the time of this study, but it was represented by other genes. A partial LSU sequence was obtained for one Mycelia sterilia A-Tan isolate (Accession no. KP137621). A Megablast search of this sequence yielded the two closest hits as unidentified isolates, with the third highest hit "*Muyocopron* sp. MFLU (CC) 10-0041," Accession no. JQ036230, with 95% sequence identity and 65% query coverage, supporting the possibility that this morphotype represents *Muyocopron smilacis*.

The shape of the endophytic rank abundance plot of presence/absence data (Fig. 4a) showed strong dominance, and a distinction could be made between dominant, intermediate, and rare species. In the corresponding epiphytic plot and combined plots, the curve was fairly smooth with no obvious transition from dominant to intermediate or to rare species.

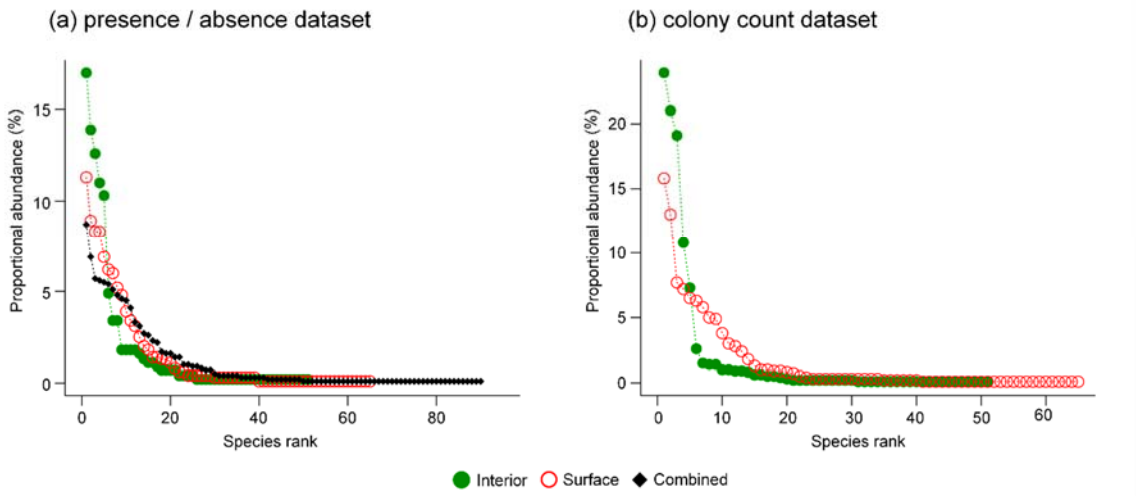

Fig. III-4. Rank abundance plots

Rank abundance plots based on samples from different portions of the plant habitat (surface, interior, and combined) and different methods of quantifying abundance. The presence/absence dataset (**a**) is based on presence counts at the 1-cm scale. The colony count dataset (**b**) is based on total counts from tissue fragments divided into 12 pieces, or tape cut into 8 pieces. No combined curve is shown for the colony count dataset as the number of possible counts was different between surface and interior; however, the individual datasets are comparable because proportional abundance rather than actual abundance is shown

The curves generated by using colony count data (Fig. 4b) showed broadly similar patterns. A long tail of rare species remained in all plots, including the combined. The endophytic assemblage contained 26 singletons (51 % of spp.), the surface also contained 26 (40 % of spp.), and the combined dataset 41 singletons (46 % of spp.). Examination of the species lists showed that in a few cases singletons in one habitat were frequent in the other (e.g. some of the *Coniothyrium*-like spp. common on the surface were singletons in interior, as well as one *Pestalotiopsis* sp.). It was more often the case though that rare species were rare in both habitats, and many were singletons in one habitat but absent from the other.

3.2 Objective 2

In the spatial analysis, the first Mantel test considering every stem separately indicated that only epiphytic fungal assemblages were significantly correlated spatially along the 160 m of greenbrier thicket (surface R=0.42, p=0.0001; interior R= 0.16, p=0.07). The second Mantel test on the more aggregated dataset (in which stem pairs taken from within 1 m of each other were pooled) led to an even stronger epiphytic correlation in terms of R value, while correlation in endophytic assemblages remained insignificant (surface R=0.60, p= 0.004; interior R=0.26, p=0.10) (Fig. 5).

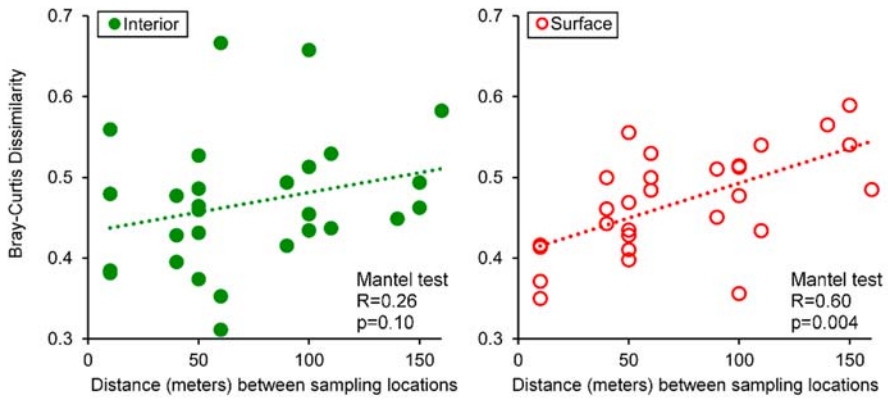

Fig. III-5. Relationship between spatial distance and dissimilarity of fungal assemblages at local scale

Data from each pair of stems (stems A & B) taken from within 1 m of each other was combined to create a pooled epiphytic or endophytic assemblage at transect locations 0, 10, 50, 60, 100, 110, 150, 160 m; linear regression line is included

3.3 Objective 3

Since frequencies among the basal five samples per stem were affected by stem position (see next section), correlative relationships were tested using only the five samples cut down from the tip. All significant correlations were positive. Mycelia sterilia A-Tan was positively correlated with two endophytes, *C. boninense* (tau=0.34, *p*=.0003), and *Phyllosticta* sp. 1 (tau= 0.30, *p*=.003), and with three epiphytes, *Coniothyrium-like* sp. 1A (tau=0.29, *p*=.008), *Pestalotiopsis* sp. 1 (tau=0.29, *p*=.005), *Pestalotiopsis* sp. 2A (tau=0.27, *p*=0.02). Two dominant endophytes, *Phyllosticta* sp. 1 and *C. boninense* showed a significant positive correlation (tau=0.38, *p*=.00002). The epiphyte *Coniothyrium*-like sp. 1B was positively correlated to the epiphyte *Pestalotiopsis* sp. 1 (tau= 0.31, *p*=.003), and cross-over species *A. pullulans* (tau=0.26, *p*=0.04). The chi-square test showed that species abundances were significantly ($p < 0.001$) dependent on which plant they were sampled from, so that 1-cm samples from the same plant cannot be

considered independent. As a result, observed correlations could be caused by proximity (autocorrelation) of samples.

3.4 Objective 4

By plotting the average number of dominant endophyte colonies at each stem position, a striking pattern emerged in which *C. boninense, Phomopsis* sp. 1, and *A. pullulans* all appeared to exist in different gradients of abundance that varied with stem position (Fig. 6). *C. boninense* was most abundant in the lowest segment sampled (3 cm from the base), while *Phomopsis* sp. 1 peaked at the third segment (33 cm from the base), and *A. pullulans* was most abundant at middle length, furthest from the base or the tip of the stems. *Phyllosticta* sp. 1 and Mycelia sterilia A-Tan were more evenly distributed across the length of the stem. The Kruskal-Wallis test showed a very significant effect of stem position on colony abundance for *C. boninense (P* =1.9x10-10) and *Phomopsis* sp. 1 (P= 4.3x10-7), and was just significant for *A. pullulans (P*= 0.049). For epiphytic fungi, while some vague patterns emerged, high and low values tended to be scattered at different heights without the strong trends seen in endophytes, so patterns in epiphytes were not investigated further.

To examine the consistency of the endophytic pattern, colony abundance of *C. boninense, Phomopsis* sp. 1, and *A. pullulans* were plotted across all individual 1-cm samples for each stem (Fig. 7). In the majority of stems, an initial spike in *C. boninense* at the basal-most segment was followed immediately by a spike in *Phomopsis* sp. 1. *A. pullulans* appeared to be more subject to variation in abundance across the site, but where it was abundant it favored the middle regions of the stems.

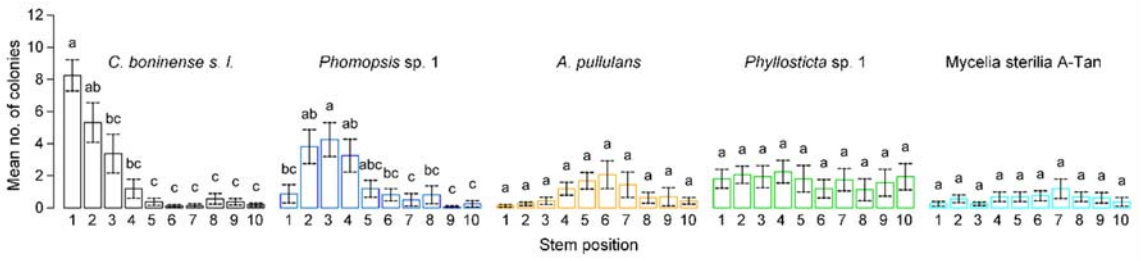

Fig. III-6. Dominant endophytes' mean frequency at different stem positions

Positions showing any of the same letters above the bar were not significantly different according to Kruskal-Wallis pairwise test, p=0.05 significance level. The error bars represent standard error. 0-12 colony counts per species were possible as presence/absence counts at each position sampled. *[Legend for Stem Positions:* Position 1=3 cm from base, 2=18 cm from base, 3=33 cm from base, 4=48 cm from base, 5=63 cm from base, 6=63 cm from tip, 7=48 cm from tip, 8=33 cm from tip, 9=18 cm from tip, 10=3 cm from tip.]

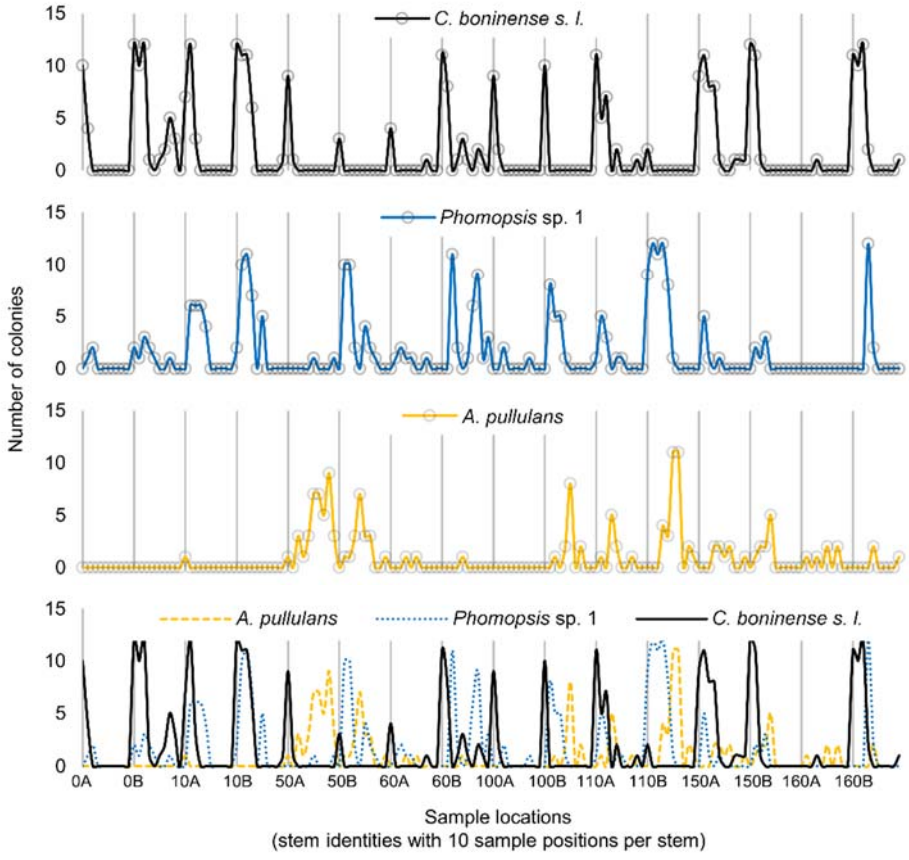

Fig. III-7. Niche partitioning along stem axis, as recorded in all 16 stems

Individual colony counts in all 160 samples are visualized above. Stem identities (indicated on the *x-axis)* are coded by location (in meters) along the transect, and stem A or B at each sampling location. Each vertical gridline represents the basal-most 1-cm segment of a stem, and is followed by 9 increasingly higher stem positions running from left to right until the next gridline is reached

Transitions where the main stem axis ended and was replaced by a side shoot were re-examined in light of these abundance patterns. Plant 60B showed an unusual second group of peaks in *C. boninense* and *Phomopsis* sp. 1 (Fig. 7) that seemed to correspond to the axis switch in that plant, but in many other plants there was no connection seen between axis switches (or lack thereof) and colony abundance patterns. (The list of axis switches focusing on the basal five stem segments is as follows: Plant 60B between the 3rd and 4th segments from the base; Plant 100B starting between the 4th and 5th segments from the base and many higher; Plant 110B between the 1st and 2nd segments from the base, then several after the 5th segment from the base; Plants 50A, 110A, and 150B had transitions that only affected the five segments cut from the tip portion).

Finally, it was observed that a zone of inhibition type antibiosis occurred very frequently and very consistently as fast growing *Phomopsis* sp. 1 colonies were inhibited when approaching slow growing colonies of *C. boninense*.

4. Discussion

4.1 Sampling methods justification

The sampling design of this study was aimed at gathering a large quantity of data from a small sampling area while limiting environmental variables in order to detect subtle spatial aspects of the fungal community. While it would have been possible to gain a better picture of greenbrier's total associated mycoflora by sampling across a broader range of geography, seasons, tissue types, and using additional isolation methods, such a

reallocation of resources would have likely sacrificed the detailed spatial information needed for the study's objectives. Due to this restricted scope of sampling, and particularly to reliance on a single medium and culture-dependent approach for isolation, characterization of greenbrier's biodiversity must be considered incomplete (Unterseher and Schnittler 2009; Prior et al. 2014). While biodiversity information was quantified, I considered this aspect as secondary to the overall objectives of this study.

As this study is part of a project to classify dominant members of greenbrier's associated mycoflora morphologically and using multi-gene phylogenies (to be published separately), a culture-based sampling approach was taken. Malt extract agar was chosen as the medium for isolation because it has been shown in previous studies to yield diverse endophytic isolates (Frohlich and Hyde 1999). It was also noted in preliminary experiments that compared to potato dextrose agar, colonies on MEA showed slower growth and greater distinguishing characteristics in terms of color of exudates, colony color and morphology. While a culture-dependent sampling bias may have influenced the species observed, any bias should apply similarly to surface and endophytic samples allowing for comparison of spatial structure and other community characteristics.

The methodology used in sampling stem surfaces was developed after considering that methods used in previous studies might not cleanly separate endophytes and epiphytes. For example, the technique often used in previous studies of serially washing then plating unsterilized tissue cannot be expected to prevent endophytes from growing out of unsterilized tissue if the endophytes are competitive, or if the tissue is sparsely colonized on the surface. This problem might be partially resolved by the approach of

some researchers (e.g. Santamaria and Bayman 2005; Kharwar et al. 2010) to place washed plant tissues on media for 1 h to facilitate growth of epiphytes from the surface, then removing the plant tissue from the media. However, this may have its own disadvantages in that it may exclude slower growing surface species, or those with a short dormancy period before emergence. Besides failing to inventory certain species, this effect could compromise any comparison of surface to endophytic diversity.

The use of adhesive tape as described in the methods to sample epiphytic fungi should be more effective in keeping purely endophytic fungi completely out of surface samples, while still allowing slow growing surface species to grow out from the tape. In addition, this method allowed us to test for correlative evidence of interaction between endophytes and epiphytes, since both groups were sampled from the same tissue segments. Although it has been suggested that these groups may interact (Santamaria and Bayman 2005), previous sampling methods always involved sampling different tissue segments for both groups.

4.2 Epiphytic vs. endophytic fungal assemblages

The results supported a view of endophytic and epiphytic assemblages more as different groups (Fig. 2, Table 1, Table 2) than as a single group with different preferences for one or the other habitat. Although a few species showed a small amount of overlap, and *A. pullulans* a large overlap, in utilization of both habitats, the overall picture was one of strong sorting across the cutaneous barrier. This is in agreement with previous culture-based comparative studies that have found as a general rule that abundant endophytic species were rare to non-existent in surface samples and the reverse

was also true (e.g. Santamaria and Bayman 2005; Osono and Mori 2004 and references cited therein; but see Kharwar et al. 2010), suggesting that the endophytic and epiphytic lifestyles involve different sets of adaptations.

The degree of overlap between surface and interior assemblages measured as Sorensen's QS of 0.45 is at the high end of the range of values from previous studies as compiled by Osono and Mori (2004). If Sorensen's QS is calculated using average of individual stems rather than the whole dataset, the value is 0.26, more towards the middle of the range. Thus the overlap between these groups in greenbrier stem communities can be considered typical to high compared to previous work (although previous studies all compared assemblages of leaves not stems). As Sorensen's QS was affected by sample size, the Bray-Curtis similarity measure (0.19 for stem average, 0.22 for the whole dataset) might be a better metric for future comparisons across studies as it accounts for differences in abundance, has many desirable qualities compared to other indices (Magurran 2004), and appears reasonably robust to sample size.

The finding that hyphomycetes were proportionally more abundant on the surface (Fig. 3) is in agreement with observations of previous researchers that ubiquitous hyphomycetes such as *Aureobasidum, Botrytis, Alternaria, Cladosporium,* and *Epicoccum* spp. tend to dominate plant surfaces (Legault et al. 1989a, b). The two most frequently observed surface hyphomycetes of greenbrier stems were *A. pullulans* and *C. cladosporioides*. This is in agreement with a recent study of fungal epiphytes (Flessa et al. 2012) in which it was shown that *A. pullulans* and *C. cladosporioides* may comprise a core of dominant species associated with dark pigmented hyphal growth on plant surfaces

found across taxonomically diverse host substrate, followed by species of *Phoma, Alternaria, Penicillium*, and some unknown taxa. Another species that was frequent and exclusively epiphytic on greenbrier, *T. myrti,* is also likely to be associated with dark hyphal growth as colonies were highly melanized, and the genus *Tripospermum* has been found associated with true sooty molds of *Citrus* spp. (Reynolds 1999) of longan (Serrato-Diaz et al. 2010), and with sooty blotch disease of apple (Wrona and Grabowski 2004). T *myrti* was classified by Revay and Gonczol (2011) as a member of the "canopy fungi," or "terrestrial aquatic hyphomycetes" sensu Ando (1992), an ecological grouping of fungi that produce mostly branched conidia and are found in rainwater that has passed through living trees. It has been observed consistently in collections of stemflow and canopy throughfall from a wide variety of tree species, from gutter water, from live twigs incubated in water, and has been observed sporulating on leaves (Gonczol and Revay 2004, 2006; Revay and Gonczol 2011). Whether regions of dark pigmented epiphytic fungi have a very different species composition than undarkened regions of epiphytic fungal growth is not known.

While there were proportionally more hyphomycetes on the surface of stems, this should not obscure the fact that coelomycetes were still the most common growth form observed in the surface group, just as in the interior. These were coelomycetes in commonly encountered plant-associated genera that are currently difficult to identify to species, such as *Pestalotiopsis, Phoma,* and *Coniothyrium-like* species, making their level of host-specificity or host-preference difficult to judge. Three *Pestalotiopsis* spp. (i.e. morphotypes) were found abundantly on the surface of greenbrier stems, while absent to rare in the interior (Table 2), similar to reports from other plant species. For example, in

Camellia japonica leaves (Osono 2008), *Swida controversa* leaves (Osono et al. 2004), *Fagus crenata* leaves (Osono 2002), and *Coffea arabica* leaves (Santamaria and Bayman 2005), past studies have found that one or multiple *Pestalotiopsis* spp. were dominant on leaf surfaces but absent to very rare from surface sterilized leaves. While reports of *Pestalotiopsis* as an endophyte also are frequent in the literature (e.g. Gazis and Chaverri 2010; Wei et al. 2007; Mariano et al. 1997), no comparative studies looking at surface and interior communities have ever shown a species of *Pestalotiopsis* to be predominantly endophytic.

Several *Coniothyrium-like* spp. and a *Phoma*-like species were mostly associated with epiphytic growth but were also found in low numbers in surface sterilized samples. This pattern suggests that these might be primary surface scavengers that are weakly parasitic or opportunistic colonists of wounds. These types of species could be more beneficial to the plant than latent endophytes in a bioprotective sense, in that they may occupy a potential weak point in the plant's defenses and may effectively exclude colonization of the plant by more pathogenic fungal species. *Coniothyrium*-like species are known surface colonizers of twigs and leaves of multiple plant genera and include both parasites of plants and hyperparasites of fungi (Damm et al. 2008). Although predominantly associated with surfaces, *Coniothyrium* can also be endophytic (Cabral 1985).

Among the endophytic group, the coelomycetes *Phomopsis, Colletotrichum,* and *Phyllosticta* were found to be leading genera in greenbrier stems, which is not surprising. The literature shows that in endophytic surveys of woody perennials, *Phomopsis* is

commonly isolated from bark and shoots, while *Colletotrichum* and *Phyllosticta* are commonly isolated from foliage (Stone et al. 2004). Greenbrier stems fall into a middle category between shoots and leaves because they are green and photosynthetic but also have a woody character. The complete lack of these species on the surface raises the question of whether there is some trade-off involved, so that specialization in one habitat may preclude success in the other. If there is some benefit in exploiting either the surface or interior habitat exclusively, then it does not seem to apply to *A. pullulans*, a species that appeared in high frequency in both endophytic and epiphytic spheres. *A. pullulans* colonies isolated from the surface of the plant differed in appearance from those isolated from surface sterilized tissue, suggesting they might be different populations or sub-species, or that they possess physiological plasticity that allows them to adapt to both interior and exterior habitats by expressing different genes. Pugh and Buckley (1971) suggested that *A. pullulans* may use endophytic space as a temporary refuge from competition, in an otherwise epiphytic lifestyle.

The predominantly endophytic morphotype, Mycelia sterilia A-Tan, was another occasional crossover species found in low but significant numbers on the surface (5% surface frequency, 9 of 160 samples). This is suspected to be the parasite *Muyocopron smilacis* based on spermatia morphology and LSU sequence. Its occasional surface presence might be explained by a parasitic lifestyle of active growth rather than latent colonization, perhaps making it more likely to cross the interior to surface barrier. Clavicipitaceous *Epichloe* endophytes of grasses, which actively grow within tissues, are also known to grow across surfaces to form epiphyllous nets (Moy et al. 2000).

Some similarities were seen in the fungal endophytic community in *S. rotundifolia* and that of *Heterosmilax japonica* stems, the only other member of the Smilacaceae for which published data are available relating to endophytic infection (Gao et al. 2005). In that study, an ITS clone library was generated containing 92 clones, of which 14.1% clustered with *Phomopsis,* 10.8% with *Glomerella/Colletotrichum,* 3.3% in Botryosphaeriaceae (the family containing *Botryosphaeria* and *Phyllosticta),* 3.3% with *A. pullulans,* 1.1% with red yeasts *Sporobolomyces* and *Rhodotorula,* and 1.1% with *Cladosporium* (red yeasts and *Cladosporium* were epiphytes in the present study). One major difference from my results was that 22.8 % of the ITS clones clustered with *Mycosphaerella,* which was not detected in my study. However, its presence is still possible as it may have been among the rare non-sporulating species that were not sequenced. Moreover, in light of evidence from recent studies of a strong seasonal influence in endophytic communities (Mishra et al. 2012; Persoh 2013), *Mycosphaerella* could potentially be common in other seasons.

4.3 Comparing diversity and rank abundance plots

While the species richness data was arguably ambiguous since the species richness curve was still rising steeply (Fig. 1a), the Shannon exponential had begun to plateau in the sampling curve, showing an epiphytic assemblage that was more diverse (Fig. 1b). Shannon exponential can be thought of as the number of species present if all species were equally common, making it an intuitively meaningful index for comparison of diversity between two environments (Magurran 2004), as well as one that weights rare and common species equally (Jost 2006). Although sampling was not exhaustive, the available evidence here suggests a more diverse epiphytic community. However,

sampling in other seasons, tissue types, and sites would be useful to present a more rounded view of the relative diversity of these assemblages in *S. rotundifolia*.

The finding of greater observed species richness in the epiphytic sphere (51 interior, 65 surface spp.) is in keeping with the general trend observed in other temperate region studies of woody plants. As tabulated by Osono and Mori (2004), in leaves of 5 of 7 tree species examined, epiphytic species richness was > 2x endophytic richness (*Eucalyptus viminalis, Swida controversa, Fagus crenata, Pinus banksiana, Pinus resinosa),* in one it was slightly greater, *(Nothofagus crenata;* 14 interior, 19 surface spp.), and in one was approximately equal *(Populus tremuloides;* 22 interior, 20 surface spp.). In a later study *Camellia japonica* leaves also yielded higher epiphytic richness (44 interior, 52 surface species) (Osono 2008). Most of these studies were in temperate to cool-temperate climate zones, with the exception of the study of *Populus tremuloides* (Wildman and Parkinson 1979), which took place in a subarctic climate. Studies in tropical to subtropical climate zones have shown more equal species richness between interior and surface, as seen in studies of *Coffea arabica* leaves in Puerto Rico (63 interior, 66 surface spp.), *Cocos nucifera* leaves in Brazil (60 interior, 61 surface spp.), and *Eucalyptus citriodora* leaves in India (20 interior, 22 surface spp.) (Santamaria and Bayman 2005; Mariano et al. 1997; Kharwar et al. 2010). More comparative data from tropical to sub-tropical as well as subarctic climates would be useful to determine if consistently higher epiphytic diversity is a characteristic of temperate climates alone.

The rank abundance plots (Fig. 4a,b), which show epiphytic curves descending more gradually than the endophytic curves between common and rare species, further

support a view of an epiphytic assemblage that was both more species rich and more even than the endophytic community. If species abundances or presence counts roughly approximate resource use within an environment (Whittaker 1965), then the rank abundance plots suggest that the bulk of resources of the interior plant tissues are divided up by far fewer species. A niche-based explanation (Whittaker 1965) seems compelling in addressing the comparative patterns seen here, but first a clear definition of niche is needed.

Hutchinson (1957) defined niche as the resources and environmental conditions necessary for each organism's survival and reproduction. In this definition, any number of environmental resources and conditions comprise axes making up an abstract hypervolume that is niche space; each point in niche space corresponds to any number of points in physical space (Colwell and Rangel 2009). However, certain distinctions describing organisms themselves fall outside of the Hutchinsonian framework of resource and conditions, such as nocturnal vs. diurnal animals, and very small vs. very large organisms with entirely different metabolic requirements (Hutchinson 1957). These might be described as categorical or other types of non-continuous axes (Geange et al. 2011), or as different "modes of life," a descriptive phrase used by Gause (1934) relating to niche (derived from Elton's niche concept, 1927). The Hutchinsonian concept, originally developed with phytoplankton in mind (Hutchinson 1978), is probably best applied within, rather than between, particular modes of life.

Endophytism and surface existence might reasonably be considered two different modes of life based on the strong division seen in most species association with one or

the other habitat. The effect of combining these two modes of life in the combined rank abundance plot (Fig. 4a) is to generate a more even community than the two that it came from. Perhaps the surface community is also more even because more modes of life intersect with the surface lifestyle than the endophytic, including different reproductive strategies and life-cycles.

There are reasons to expect that this may be true. Endophytic sampling, by targeting asymptomatic tissue, eliminates interior colonists that might reproduce via the destruction of plant tissue, and by doing so may eliminate many life-strategies of interior colonists. Reproduction of fungi that are situated on a living plant surface is less likely to be destructive to the plant, as there is no need to destroy or disrupt plant tissue to generate reproductive structures. Dickinson (1965) showed that epiphytes could be divided into those that reproduced on the living leaf surface and those that grew on the living leaf, but only reproduced after leaf senescence. The greater contingent of hyphomycetes on the surface (Fig. 3) may reflect many of these species that reproduce on living plants without a need to wait for, or induce, tissue senescence (logically, hyphomycetes might be expected to need fewer resources and less time than coelomycetes to complete their life cycles since hyphomycetes produce conidia directly without investment in the production of conidiomata). Though even within the growth form of coelomycetes, multiple strategies probably exist on plant surfaces. For example, Osono (2002) isolated three frequent *Pestalotiopsis* spp. from the surface of *Fagus crenata* leaves, one of which was most frequent on living leaves, one of which was most frequent on senescing leaves, and one that was equally frequent in living, senescent, freshly fallen, and decomposing leaves. In conclusion, rather than simply assuming that the surface community is more

diverse because it is easier to enter, a better explanation for this diversity may be that the surface community, as sampled, encompasses more fungal modes of life.

4.4 Spatial correlations, evidence for limited dispersal

The observed spatial correlation in epiphytic assemblages (Fig. 5) most likely represents the effects of dispersal limitation. However, the potential effect of the surrounding environment should also be considered. Heterogeneity along the transect may result from leachates from surrounding trees, shade and moisture that may put different selective pressures on fungal populations at different locations. While such selective pressures might also lead to spatial correlation, such factors should only influence nearby locations such as those at the scale of 10 m apart. Since spatial correlation of communities was at the entire 160-m scale (Fig. 5), and there was no continuous variable along the transect that can explain this, imperfect dispersal seems the likely explanation. One other way that environmental heterogeneity might come into play is as a source for epiphytic propagules of generalists. If this is the case, then the environment is acting not as an agent for selection, but as a source of heterogeneity in dispersal; this is probable and is not at odds with a conclusion that dispersal is limited.

In contrast to epiphytes, endophytic assemblages did not show spatial correlation. Yet there is no biological reason to believe that the mechanics of endophytic dispersal of propagules should be more uniform than epiphytic, since both groups are dominated by genera of rain-dispersed coelomycetes (Bilgrami 1963; Parker and Ramsdell 1977; Yang et al. 1990; Carnegie 1980), and other modes of dispersal such as insect vectors (Devarajan and Suyanarayanan 2006) could also apply to either group. Instead, I

hypothesize that longer persistence of endophytic colonies inside the more stable internal plant environment might effectively make dispersal more uniform across the plot as propagules reach all plant surfaces over time. Evidence for long-term persistence of endophytes is suggested by observations that endophytic colonization frequency increases with age of tissue (Stone 1987; Rodrigues 1994; Fisher et al. 1986) (though residence time of endophytic and epiphytic infections in plants is unknown so this is only speculative until empirical data are available). Perhaps more importantly, high differential fitness of endophytic species based on internal plant factors is suggested by the preference of different species for varying positions in stem height. Such selectivity by internal plant factors might help to negate any stochastic effects that could lead to site-wide spatial correlation.

In another study of spatial structure at the local scale, Cordier et al. (2012) found that dissimilarity of phyllosphere communities of whole beech trees *(Fagus sylvatica)* on a scale of a few meters to 300 m apart was correlated to genetic distance but not to spatial distance. At the level of groups of leaves within the same tree there was correlation by spatial distance. Since Cordier et al. (2012) studied endophytes and epiphytes in the same molecular samples, it is not clear which group was driving this dynamic. It is not necessarily in conflict with my results, as both studies found evidence of spatial correlation. It can be concluded that spatial correlation of fungal assemblages does exist at the local scale, but may be negated in different groups by the effects of genetics or other selective effects. I do not know the degree of genetic similarity in sampled greenbrier stems; genetic similarity in the host could have had a strong influence on endophytic assemblages in the greenbrier samples.

4.5 Inter-species correlations and evidence suggesting species interactions

Despite the non-independence of samples taken from the same plant, the fact that five species were positively associated with a suspected parasite (Mycelia sterilia A-Tan/*Muyocopron)* suggests that these results were not simply an artifact of spatial autocorrelation between samples. These positive associations might be explained in that parasitic activity by this species could make tissue more conducive to colonization and/or resource scavenging by other species. Alternatively, some common aspect of the stem tissue may make it suitable for colonization by the parasite along with the other species.

It is also notable that correlative tests showed no interaction of species that were strictly interior endophytes with species that were strictly exterior epiphytes. Species that were confined within the same microhabitat showed significant correlations with each other (interior: *C. boninense* with *Phyllosticta* 1, surface: *Pestalotiopsis* sp. 1 with *Coniothyrium-like* sp. 1B). Also, species that were not entirely confined to the surface or the interior (i.e. *A. pullulans*, Mycelia sterilia A-Tan) showed interactions with those that were strictly confined to the interior or exterior. In other words, the correlative data only supported possible interactions between species that might potentially occupy the same physical space; no evidence could be seen suggesting that chemical or other interferences across the epidermal barrier could influence abundances of different species.

Finally, although no negative correlations were noted, some species may be present as survival structures without active growth, masking the effect of competition (Pugh and Buckley 1971). In addition, the 1-cm scale of correlative testing may have

been too large to detect competitive effects. Because of this, the possibility of competitive interactions between these species should not be discounted.

4.6 Niche partitioning by stem position

The pattern of stem height preference among some endophytes (Figs. 6, 7) was the most unexpected finding in this study, as this pattern has not been observed in other plant-endophyte systems to my knowledge. This pattern might be compared to 'tissue preference' sometimes reported in endophyte studies (e.g. Wang and Guo 2007; Wearn et al. 2012; Mishra et al. 2012). But while tissue preference might reasonably be attributed to differential binding and germination of spores on different types of surfaces, differences in the stem surface at 15 cm intervals are probably minimal to nonexistent, suggesting that either competition between endophytes or another internal variable is responsible.

There is some precedent for such a pattern in studies of decomposers. As reviewed in detail by Hudson (1968), wet chamber studies of fungal succession from flowering to decomposition of the grasses *Dactylis glomerata* (Webster 1956, 1957), *Agropyron repens* (Hudson and Webster 1958), and the umbelliferous herb *Heracleum sphondylium* (Yadav 1966) revealed that certain fungi were constrained to appear only in the lower or upper nodes of stems. It is interesting to note that a recent endophytic survey of *Dactylis glomerata* (Marquez et al. 2007) revealed that several primary decomposers identified in Webster's (1956, 1957) studies were present as endophytes. Although it was not tested in that study, it would be worthwhile to know whether endophytic species abundances in the plants were height dependent.

Causes for height partitioning of decomposers in those studies of succession were speculated to relate to nutrient content of substrate, moisture in the air, or moisture in stems and leaves as they senesced (Hudson 1968). These explanations do not apply as well to endophytic colonization, since moisture content available within tissue of living stems should be less variable than that of dying stems. Nutrient differences and air moisture content also are factors more likely to play a role in active growth and competition in dead tissue as opposed to latent colonization.

One plausible explanation for the observed pattern could be a gradient of multiple plant-produced secondary metabolites that vary along the length of the stem, and to which the colonizing endophytes have different tolerances in their ability to survive (much as in a Hutchinsonian model of niche). If inhibitory phytochemicals are involved, some increasing, while others decrease along the length of the stem, it could keep species abundances highest within zones to which they are best adapted. The *Smilax* genus is known to be rich in steroidal saponins for which the rhizome of some *Smilax* species are harvested and used medicinally (Challinor et al. 2012). While there is no evidence one way or the other for a phytochemical gradient that might produce different adaptive zones in *S. rotundifolia* stems, several studies demonstrate that it is not unlikely.

For example, in *Lilium longiflorum,* another monocot rich in steroidal saponins, Munafo and Gianfagna (2011) found significant differences in the concentrations of two furostanol saponins and two steroidal glycoalkaloids between upper and lower stems. While most of these phytochemicals showed highest concentrations in the lower stem, one of them showed the opposite trend. They also found that the proportion of steroidal

glycoalkaloids to furostanol saponins in upper stems was more similar to that of leaves than that of lower stems. In a similar example, Fischer et al. (2011), studying leaf chemistry of sweet basil (*Ocimum basilicum*), concluded that essential oil profiles were dependent on the position of the leaf on the stem independent of leaf age. The lowest leaves on the main stem axis contained a high percentage of methyl eugenol, which decreased to near zero in higher leaves. Three other essential oils went from very low in the most basal leaves to varying percentages (mostly higher) at other positions. In lateral shoots a similar gradient was observed starting at the base of these shoots.

The idea that plant chemistry affects endophytic colonization is not new and has been cited in many papers as a possible factor affecting endophytic biology and differential host affinity (e.g. Van Bael et al. 2005; Espinosa-Garcia et al. 1996), including possible effects on germination, penetration, and persistence in living leaves (Arnold and Herre 2003). Saunders and Kohn (2008) provided some of the best evidence to date of the influence of plant defensive compounds on endophytic communities and suggested that defense compounds altered competitive interactions between fungi in that study. In a recent study, Balint et al. (2013) found significant differences in endophytic communities based on genotype in common garden experiments of balsam poplar; they suggested that differences could be caused by genotype mediated differences in essential oils. The differentiation observed in greenbrier at the scale of tens of centimeters gives another reason to suspect plant chemistry as a potentially powerful variable shaping endophytic communities.

As a working hypothesis, I suggest considering a stress-tolerance model of endophytic communities, in which tolerance of constituent plant-produced secondary metabolites is a dominant factor mediating successful colonization and survival of endophytic propagules, leading to the frequently observed pattern of dominance by a small number of endophytic species in most sampled substrates. Under this model, a tremendous number of fungal species are probably adapted to enter living plant tissue of nearly any plant, a fact already evident by the large number of total species frequently observed in endophytic surveys. While various endophytes may enter the plant at similar rates, those well adapted to survive the chemical milieu (i.e. with a low mortality rate) will steadily rise above the others in abundance, with differential mortality taking place on the order of days, weeks, or months among co-dominant species with subtle differences in adaptation.

In support of this idea, several studies suggest the unstable nature of endophytic infections. Osono (2008) showed that in evergreen *Camellia japonica* leaves, abundance of various endophytic species were subject to seasonal fluctuations across all leaf age classes from first year to 3-year-old leaves. Tadych et al. (2012) demonstrated a succession of fungi in fruits of cranberry during several weeks of development, with species present early in development disappearing as fruits matured. Such seasonal and developmental fluctuation in endophytes in the same tissues demonstrates that infections are not permanent once made, but may disappear due to death of the endophyte, allowing for potential differences in mortality rate to play out between co-dominant species in slightly varying environments (e.g. leaf vs. shoot, or different heights along the stem, different developmental stages of tissue). As the non-permanence of endophytic

infections is strongly inferred by these studies and others (Persoh 2013), more research is clearly needed on the particular factors inducing mortality (or non-detection) of infections, and the charting of survival curves for endophytic infections (and for epiphytic infections as well).

Arnold and Herre (2003) showed that endophyte-free plants could be grown indoors and inoculated with combinations of endophytes for controlled experiments. Controlled experiments with initially endophyte-free plants could reveal whether survival or death of endophytic colonies, especially those best adapted to different tissues, is determined instantaneously or over more gradual lengths of time. Additionally, inoculation of endophyte-free greenbrier plants with the dominant endophytes either alone or in combinations could help determine whether the observed height partitioning of the endophytes in greenbrier is caused by interactions with the plant alone or whether there are also competitive mechanisms involved within living plants. Direct competition need not be necessary, as endophytes can affect the production of volatiles and probably other secondary compounds produced by plants (Mucciarelli et al. 2007).

4.7 Conclusions

In summary, it has been demonstrated that largely separate fungal assemblages occupy surface and interior spaces of greenbrier stems. Endophytic niche partitioning by height, and antibiosis observed in vitro, suggests that in the endophytic sphere several dominant species with similar lifestyles are competing but are optimized for survival under slightly different conditions, as in a Hutchinsonian model of niche. Such niche partitioning also suggests that strong selectivity exists for endophytic organisms at a fine

scale of internal differences, and so perhaps strong selectivity by other unknown aspects of the substrate may have influenced the lack of spatial structure seen in endophytic assemblages at the local scale of 160 m. Among epiphytes on the other hand, the spatial correlation that was evident at this scale suggests that chance dispersal limitation could play more of a role in structuring their community, and that individual differences in stem genetics and/or physiology play lesser roles. More research into the niche requirements and life cycles of co-occurring phyllosphere fungi would help to advance understanding of how the micro-fungal community is assembled.

Some of the results of this study, including the low overlap of epiphytic and endophytic communities, and higher epiphytic species richness, support a consensus with previous studies in temperate climates. The more novel findings—including that spatial structure was present in the epiphytic but not endophytic community, that correlative relationships between endophytes and epiphytes are absent, and the presence of height-mediated niche partitioning—should be replicated in other seasons, locations and plant species to determine if they can be generalized. While it can be challenging to simultaneously study multiple groups of plant-associated organisms, such work will hopefully be a first step towards generating hypotheses and developing a framework for understanding the whole plant microbial community. As emphasized by Wearn et al. (2012), to advance plant-microbial ecology it is important to move beyond single group perspective towards a greater understanding of the plant ecosystem.

Chapter IV. The summer study: the influence of season, tissue-type, and geographic locality on epiphytic and endophytic fungal communities of greenbrier

Abstract

While endophytic assemblages have been well studied from the perspective of geographical variation, tissue specificity, and community structure, epiphytic fungal assemblages seldom have been. I studied both groups simultaneously, in leaves and stems of the woody vine, *Smilax rotundifolia*, to incorporate epiphytic fungi better into understanding of the phyllosphere. Plants were sampled in nine sites spread over three different environments: the New Jersey Pine Barrens, coastal sites of Sandy Hook national park, and urban sites of Rutgers University. I predicted that epiphytes would be highly variable in different environments. I found that the combined (surface and interior) stem communities within the same environment were found to be more similar to each other than those in other environments per ANOSIM testing. While this implies an effect of locality, the dominant species were similar across the sites whether endophytic or epiphytic assemblages were considered. Epiphytic stem communities were dominated by *Aureobasidium pullulans* and three *Pestalotiopsis* species. Endophytic stem communities were dominated by *Phyllosticta* spp., *Phomopsis* sp., and several mycelia sterilia morphotypes. *Aureobasidium pullulans*, and the morphotype Mycelia sterilia B-White (which was determined to be an *Anthostomella* sp.) were found in high frequency on both surfaces and interiors of both stems and leaves. Tissue type (leaf vs. stem) affected both endophytic and epiphytic assemblages, though there was a more distinct effect on endophytes. In determining overall makeup of fungal community, I found that surface vs. interior environment was the largest determinant, followed by tissue type, and finally by location. A clear seasonal effect was also noted by comparison to the dataset of a previous study. This is the first study to demonstrate that epiphytic fungal communities are consistent host associates in geographically different locations, and demonstrating the influence of tissue type on epiphytic fungal communities.

1. Introduction

The phyllosphere, broadly defined, is the microbial habitat comprised of all of the above ground, living, tissues of plants – a highly heterogeneous environment that covers large portions of the earth's surface (Andrews & Harris 2000). The filamentous fungi found there can be usefully organized, from a sampling perspective, into the three, non-mutually exclusive, categories of (1) endophytes, (2) epiphytes, and (3) pathogens.

Pathogens are defined by their symptoms, while endophytes and epiphytes are defined as those fungi that occupy the interiors and surfaces of seemingly healthy plant tissues.

The ecological role or roles of these fungi, and their importance to living plants, is an ongoing area of investigation. Some proportion of seemingly symptomless phyllosphere fungi are probably weakly parasitic (Photita et al. 2004), converting to pathogenesis under the right conditions (Alvarez-Loayza et al. 2011). Some others, such as the mutualistic clavicipitaceous endophytes, have a special relationship with the plant, systemically growing within the tissues, and being passed vertically to offspring (Kuldau and Bacon 2008). Most remaining, non-parasitic, phyllosphere fungi are probably either (1) accidental colonists in a dead end position that will not complete the life-cycle (Petrini 1996), (2) able to complete their life-cycle on living plants (Dickinson 1965) using sparse nutrients such as plant leachates (Tukey 1971) and pollen (Fokkema 1971), or (3) as preemptive colonizers that will take part in later tissue decomposition (Chapela and Boddy 1988; Sun et al. 2011; Osono 2006; Osono and Hirose 2009; He et al. 2012; Purahong and Hyde 2011). Early access to plant tissues before senescence may be essential to the success of some species in this last group (Koide et al. 2005; Osono 2002). However, a partially saprotrophic ecological role does not preclude these fungi from affecting living plants. It has been shown in many studies that a variety of both endophytic and epiphytic species may either reduce or enhance the effects of plant disease (e.g. disease reduction - Arnold et al. 2003; Dingle and Mcgee 2003; Perello et al. 2002; Clarke et al. 2006; Ganley et al. 2008; Istifadah and McGee 2006; Raghavendra and Newcombe 2013; Lee et al. 2009; Andrews et al. 1983; disease increase - Morin et al. 1993a,b; Busby et al. 2013; different effects by different species - Kurose et al. 2012,

Ridout and Newcombe 2015, and multi-study summary in latter). Also, a reduction in insect herbivory has been attributed to taxonomically diverse foliar endophytes of *Allium* herbs (Muvea et al. 2014).

Not surprisingly, there is current interest in the manipulation of the plant microbiome, whether for the benefit of cultivated plants or biocontrol of invasive weeds (e.g. Kurose et al. 2012; Kowalski et al. 2015). Yet there are still some basic gaps in our understanding of how phyllosphere communities are organized. Although endophytes and epiphytes cohabitate the phyllosphere, endophytes are much better studied (e.g. they are considered as a mycological sub-field: “endophytology," Unterseher 2011), especially in ecological questions of biodiversity, niche delineation and community structure. Past studies have shown that the epiphytic habitat is more species rich, and largely non-overlapping with the endophytic community. But biodiversity of this group is not often studied, as epiphytes are often thought of as mainly ubiquitous saprophytes. While certain core epiphytes, such as *Aureobasidium pullulans* and *Cladosporium cladosporioides* are ubiquitous, studies have repeatedly found an abundant presence of diverse coelomycetes unidentified to species and of unknown level of host affinity. The surface phyllosphere should be more thoroughly investigated than it has been in the past, integrating the study of endophytes and epiphytes together into a more complete conceptual framework. Three areas in which epiphytic fungi have been poorly integrated into our understanding of the phyllosphere, are elements of **(1) geographic variation**, **(2) environmental niche**, and (3) **community structure**.

This study is also a follow-up, and expansion, of an earlier, single-site study, in which I examined the stem phyllosphere fungi of the woody understory shrub and climber, *Smilax rotundifolia* (common name 'roundleaf greenbrier,' referred to below simply as 'greenbrier'). Since a single location was previously studied, a natural question was whether the endophytic and epiphytic communities found in that study should be considered locally highly idiosyncratic, or could be expected to be similar in other populations of greenbrier. Geography of fungal endophytes has been studied at scales of regions (Collado et al. 1999; Gange et al. 2007; Rollinger and Langenheim 1993), different biomes (Higgins et al. 2007), and between continents in different hemispheres (Fisher et al. 1993). For endophytic communities, an expectation based on these studies is that they may vary significantly in isolation frequency, but tend to show the same dominant species in most or all sites sampled within the same region (but not in different biomes, different continents, and maybe not in the tropics). However, the literature provides very little information about the amount of geographic variation to be expected in epiphytic communities on the same host. Only a small number of studies have sampled fungal epiphytes at several locations (Santamaria and Bayman 2005; Legault et al. 1989), and even in these, understanding geographic variation was not a major part of the objectives and study design.

As a hypothesis, I predicted that epiphytic communities would be colonized by propagules from large amounts of dead, decomposing plant material found as litter and standing wood in the surrounding forest. Thus, they would be highly characteristic to the locality sampled with high turnover if sampled in different types of forests. The topic of geographic variation (within the same general region/latitude) could be easily explored

with the greenbrier plant, as I was able to locate populations of greenbrier in at least three broadly different environments within the state of New Jersey with very different vegetative assemblages – the conifer dominated Pine Barrens (Brenden T. Byrne State Forest), maritime environments of a coastal spit (Sandy Hook, Gateway National Recreation Area), and fragmented broadleaf forests of Rutgers University, New Brunswick. These three locations also fell within three of the five different climate regions defined by the Office of the State Climatologist - pine barrens, coastal, and central. (http://climate.rutgers.edu/stateclim/?section=njcp&target=NJCoverview, accessed Feb 26, 2015; (Ludlum 1983)

Our other main goal was to analyze how endophytes and epiphytes are both affected by a typically strong niche factor in endophytes, plant tissue type (Kumar and Hyde 2004). The niche is defined here as the environmental conditions to which species are adapted (Hutchinson 1957), not in the sense of life strategy or role in the community. More specifically, I am interested in those environmental conditions that favor certain species over others, as reflected in frequency or density of colonization; whether this involves competition or simply different environmental adaptations is unknown. The niches of specific endophytes, in this environmental sense, have been shown to be related to (1) plant host species (Suryanarayanan et al. 2000; Sun et al. 2012; Unterseher et al. 2007; Persoh 2013), (2) tissue/organ type (Wang and Guo 2007; Kumar and Hyde 2004; Sun et al. 2012), (4) season (Tadych et al., 2012; Mishra et al. 2012; Unterseher et al., 2007), (3) tissue age (Hata et al. 1998; Osono 2008), (6) distance from the base of a stem or leaf (Zambell and White 2014; Hata and Futai 1995), sun vs. shade (Unterseher 2007), and in at least one demonstrated case, host defense compounds (Saunders and Kohn

2009). Far fewer studies (if any) have addressed differences in the environmental niche of epiphytes showing specific organisms to thrive in conditions in which others become scarce. But in a more general sense, measures of epiphytic community similarity have been shown to be influenced by (1) host genus or higher taxonomic levels (Kembel and Mueller 2014), (2) season and leaf age (Osono 2008), (3) the concentration of aluminum, a toxic metal, (4) traits relating to leaf resource uptake strategies, (5) the growth and mortality rates of the leaves (Kembel and Mueller 2014), or similarly, annual vs. evergreen habit (Flessa et al. 2012). The issue of tissue-preference of leaves vs. stems or other plant organs has not been addressed in studies of epiphytes to my knowledge.

Furthermore, the issue of whether different tissue-preferences should be expected among endophytic fungi between subtly different tissues is not settled. One peculiarity of greenbrier stem tissue is that, as a monocot, it has a green outer stem layer with no bark. Several studies of endophytes have compared endophytic fungal communities of green, bark-less stems (or pseudostems in some monocots) to leaf tissues (Persoh 2013; Photita et al. 2001; Bussaban et al. 2001) but these conflict in their conclusions as to whether endophytes show differential preferences for the two tissue types. I predicted that tissue would influence both endophytic and epiphytic communities, since both the surface texture and internal conditions probably differ from that of stems.

This is the first study to measure the relative influence of surface vs. interior habitat, different tissues, and different locations on the phyllosphere fungal community. It is also the first study to my knowledge in which multiple localities were all sampled on

the same day, minimizing the influence of different sampling times (e.g. see Persoh 2013), so that the effect of locality could be better isolated.

Objectives were defined as:

(1) Diversity: Determine if the previously observed pattern of greater epiphytic diversity on stems is consistently observed across multiple sites and in a different season.

(2) Geography: Determine if stem microbial communities (surface, interior, combined) are significantly influenced by the sampling locale (pinelands, maritime, urban). Is geographic turnover in dominant species of the plant surface much greater than in endophytic fungi?

(3) Assess the relative influence of surface vs. interior habitat, tissue-type, season, and locality on fungal community composition and structure (seasonal comparisons were made comparing data from the previous study).

2. Materials and Methods

2.1 Sample collection and processing

Sampling sites were arranged so that there were three main localities, or 'major sites,' each of which contained three 10 x 60 m sampling plots, or 'minor sites' (Fig. 1). All sites were located within the state of New Jersey, USA, which has a temperate seasonal climate and is located in the mid-Atlantic region of the country. Minor sites were chosen that contained at least 60 meters of continuous or semi-continuous greenbrier growth. The major sites were between 38 to 77 km apart, while minor sites within the same locality ranged from 0.5-3.8 km apart. The major site 'Pine Barrens

(PB)' was in Brendan T. Byrne State Forest in Burlington County. The site 'Sandy Hook (SH)' was located in the Sandy Hook branch of the National Gateway Recreation Area of the U.S. National Park System, in Ocean County. The 'Rutgers Campus (RU)' site encompassed wooded areas of Rutgers University Cook Campus, including the old-growth Helyar Woods (site RU3), in Middlesex County.

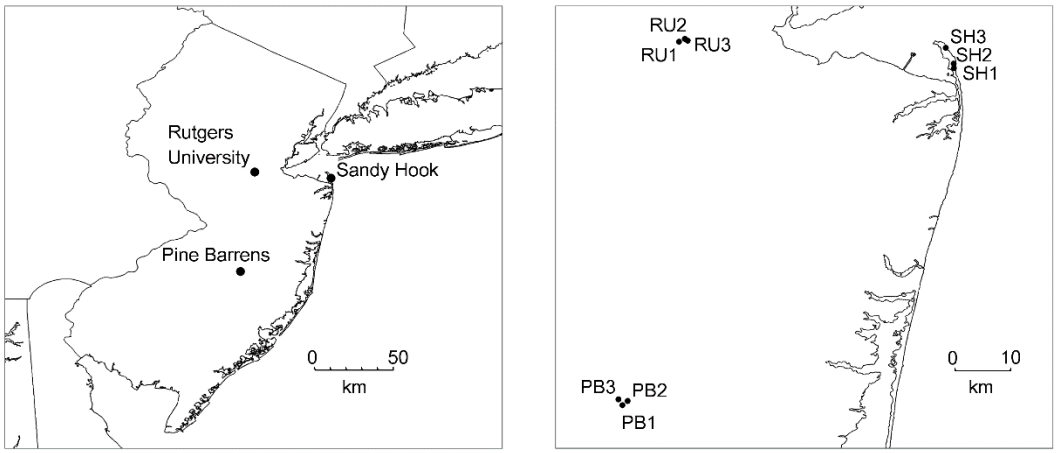

Fig. IV-1. Sampling sites

Greenbrier stems and leaves were sampled at three major sites in the state of New Jersey (USA) that have distinct vegetation and climate (left). Within each of these sites, three sampling locations (minor sites) were designated (right).

At each minor site, a 10 x 60 m plot was established at least 1 meter from any road or path, and GPS coordinates were recorded. To characterize the forest composition of each plot, every tree over 4 cm diameter at breast height was measured for diameter and the genus recorded. The composition of the shrub layer was recorded but not quantified. Soil characteristics were recorded based on GPS coordinates and using the USDA-NRCS Soil Survey Geographic (SSURGO) datasets for Burlington, Ocean, and Middlesex Counties, NJ (downloaded Aug 14, 2014, http://websoilsurvey.nrcs.usda.gov/). Meteorological data was obtained from the NOAA's National Climatic Data Center, Climate Data Online service (http://www.ncdc.noaa.gov/cdo-web/). Weather stations near each of the major sites were

chosen based on proximity and for having the needed metrics (for Pine Barrens: Indian Mills, NJ US, USC00284229, for Sandy Hook: Sandy Hook, NJ USC00287865; for Rutgers Cook Campus: any of three New Brunswick, NJ stations, USC00286055, USC00286062, USC00286053). Elevation of sites was estimated using GPS coordinates and Google Earth.

From each minor site, 6 stems and 6 leaves were collected and processed to yield 18 x 1-cm endophytic stem samples (divided into 10 fragments each), 18 x 1-cm epiphytic stem samples (divided into 8 tape strips each), 6 endophytic leaf samples (in four fragments each), and 6 epiphytic leaf samples (in 4 fragments each). Stems were marked in advance and all sampled on the same day, August 5, 2011, in dry weather. The 6 stems and 6 leaves were collected at 10 meter intervals in each plot. Leaves were not necessarily connected to the sampled vines, and those selected were as free from visible insect or disease damage as possible (at the time of sampling, most leaves had some form of damage). Plant tissues were transported back to the lab in separate plastic bags for stems and leaves and for each minor site to prevent cross-contamination. Measuring from the tip of each vine at 15-cm intervals, 3 x 4-cm lengths of stem were cut, and each of these split into a 1-cm endophytic, and a 1-cm epiphytic tissue sample. Each leaf was split down the middle into two halves, one to be processed for endophytic samples, the other for epiphytic.

Samples were refrigerated (4°C) until processed, and to eliminate bias towards major sites due to processing time, processing and isolations were made from sites in the order: PB1, SH1, RU1, PB2, SH2, RU2, PB3, SH3, RU3. Endophytic sample processing

began immediately (first stem, then leaf) and was completed within 3 days of collection in the field, while epiphytic sample processing was completed within 7 days of collection.

Endophytic stem samples were sterilized with manual shaking for 1 minute in 70% ethanol, 3 min in 0.62% sodium hypochlorite (10% Clorox), then 3 changes of sterile DI water with 1 min shaking each, then 10 s in 95% ethanol, followed by minimum 30 min drying in the hood on a sterile autoclaved tissue paper. Each stem was then cut to 10 approximately equal disks with a flame-sterilized razor, and plated together on a standard 8.5 cm petri dish of Malt Extract Agar.

For ***endophytic leaf samples***, leaves were trimmed then sterilized in the same way as the endophytic stem samples, except they were washed for only 1 min in 10% bleach. Four discs of healthy-looking tissue were punched out with a sterile 6-mm cork borer, and plated together on an MEA plate. Two were placed with the underside of the leaf on the agar, and the other two placed with the top of the leaf on the agar.

For ***epiphytic stem samples***, a prewash was done in which each of the three 1-cm cuts from the same vine were placed together in a stainless steel mesh tea ball. Six steel mesh balls representing the six plants from a single site were packed into a beaker and washed for 30 min of fast-running tap water. Stem segments were then washed individually in sterile DI water and dried; the stems were rolled across tape, and the tape plated into eight segments on MEA as described in the previous study/chapter (Zambell and White 2014).

For ***epiphytic leaf samples***, leaves were also prewashed under running tap water for 30 min (segregated by minor site). Leaves were then washed individually in sterile DI water following the stem procedure above. Tape was then drawn across a U-shaped glass rod (as with stems), then the bottom of the leaf was pressed to the tape with a sterile glass rod, leaving a leaf impression on the tape. Two discs were punched from the tape with a sterile 6-mm cork borer and plated sticky-side-down on MEA. Two additional discs were rendered by repeating this process but pressing the top of the leaf to the tape.

After 4-8 days, isolations were made, and surface-disinfected tissue segments that had no growth were transferred to new plates to avoid overgrowth. For epiphytic plates (tape), no transfers were attempted since growth tended to be faster and tape more heavily colonized. Plates were reviewed intermittently and isolations made for 6 months after. For highly common morphotypes, each morphotype was isolated only 1 time per plate, though all were noted. Isolates were examined both macroscopically for colony growth rate, color, texture, and microscopically for size and morphology of spores before designating the final morphotypes. Morphotypes of yeasts and bacteria were rare, were not scrutinized for morphology, and were not included in the final dataset. Most yeasts were likely removed in the extensive washing process (Fokkema 1991).

2.2 Analysis of stem dataset

For most analyses (unless stated otherwise), the fungal assemblages were quantified as presence counts on the scale of 1-cm of stem (i.e. only one presence count was made if a species was present on any or all of the 10 tissue slices per 1-cm stem

sample, leaf discs, or cut of tape). Frequency was calculated as (presence counts ÷ no. of 1-cm segments or leaves sampled)*100.

EstimateS Version 9.1.0 (Colwell 2013) was used to create smoothed sampling curves for species richness, treating the data as multiple sets of replicated sampling and using the 9 minor sites as the sample unit. Curves for species richness (s) were randomized using 500 bootstrap replicates without replacement using the variable 'S(est)' with 95% confidence intervals (Colwell et al. 2004). The Shannon index (H') was calculated in R v3.0.1, using package vegan 2.0-10, function *diversity{vegan}*, using the default natural log, then converted to Shannon exponential ($e^{H'}$). Pilou's evenness (J') of each site was calculated as H' / ln (species richness). Bray-Curtis similarity matrices were generated in PAST v3.01 (Hammer et al. 2001), and converted to dissimilarities where needed by subtracting from 1.

Colonization density was calculated for each site as the mean number of colonies yielded per 1-cm of stem. Analysis of Variance (ANOVA), implemented in R function aov{stats}, was used to assess whether colonization density differed between minor sites (data was approximately normally distributed). Pairwise comparisons were made using the Honestly Significant Difference (HSD) test in R package agricolae (De Mendiburu 2009), function HSD.test{agricolae}. Since the number of fragments of tape differed from that of tissue, and since sampling process was inherently different, these tests were kept separate for endophytes and epiphytes. Pearson's product-moment correlation was assessed between colonization density and forest density, and between endophytic and epiphytic colonization density, using R function cor.test{stats}.

For geographic analysis, the non-parametric test, Analysis of Similarities, or ANOSIM (Clarke 1993) was used to test whether stem assemblages were more similar within the same major site. Similarity of the minor sites was visualized using non-metric multidimensional scaling (NMDS). For both ANOSIM and NMDS, the Bray-Curtis similarity measure was used. Besides endophytic, epiphytic, and combined stem assemblages, forest (trees > 4 cm dbh) similarity of minor sites was also assessed using ANOSIM and NMDS. To weight larger trees more heavily, basal diameter/hectare of each tree species was used as the unit for the Bray-Curtis similarity in these tests.

2.3 Stem and leaf comparisons

A reduced stem dataset, 1/3 the size of the original, was also made for a more equitable comparison to the leaf dataset, since 1 leaf was sampled for every three 1-cm stem segments. The reduced dataset incorporated only the second of the three 1-cm segments (34 cm from the tip) cut from each stem. Using the reduced stem dataset and leaf dataset, each minor site (9 total) was divided into a set of (1) endophytic stem samples, (2) epiphytic stem samples, (3) endophytic leaf samples, and (4) epiphytic leaf samples for a total of 36 sample sets. ANOSIM and NMDS were performed to test for statistical support in differences between the four sample types regardless of location of sampling.

To compare community structure in different niches, a cumulative abundance plot (Magurran 2004) was generated in R package BiodiversityR, using the full leaf datasets and the reduced stem datasets for comparison. In this type of plot, species rank is plotted along the x-axis, while y-axis plots *cumulative abundance*. *Cumulative abundance* =

proportional abundance of the x-ranked species + *proportional abundance* of all species higher in rank. *Proportional abundance* = (counts of each species ÷ total counts of all species) x 100.

2.4 Seasonal comparison to previous study

The raw dataset from the earlier study/chapter (Zambell and White 2014; henceforth referred to as 'the winter study') was used to make a seasonal comparison against the summer dataset from the present study. In that study *S. rotundifolia* stems were sampled in March and early April of 2010, prior to bud break, at site RU1. The sample set from winter contained 10 x 1-cm samples along different positions of the stems and sampled 16 stems at site RU1. By comparison, the summer dataset mainly differed in that it only sampled three positions cut from the tip, and sampled 6 stems total at site RU1. To make a comparison, a modified dataset was constructed, in which only winter samples taken at stem positions 18 cm, 33 cm, and 48 cm from the tip were included. Secondly, bootstrap resampling was done using the 16-stem dataset to determine what could be expected from repeated resampling of just 6 of those 16 stems, with 95% confidence intervals. This was done using R function *sample{base},* to generate 10,000 bootstrap datasets with replacement. Presence counts of each dominant species were summed in each of the 10,000 replicates, and 95% CI for these counts was generated directly from the bootstrap data using R function *quantile{stats}*. This analysis was made on morphotypes of ≥10% frequency in either study, excluding *Phoma*-like and *Coniothyrium*-like morphotypes. Since all rare *Phoma*-like or *Coniothyrium*-like morphs in the summer study were not compared against cultures of dominant species from the winter study, seasonal comparisons were avoided for these. Also, since yeasts were rare,

and not recorded in the present study, no comparison was made to the commonly recorded (15% frequency) *Sporobolomyces*, red yeast morphotype, from the winter study.

2.5 Molecular identification

DNA sequences coding for the ribosomal RNA internal transcribed spacer (ITS) region were obtained as follows. Small cubes of agar with mycelium were cut from pure isolates on MEA or PDA, and grown in Potato Dextrose Broth (Difco) on a shaker for 1-2 weeks. Spheres of mycelium were squeezed dry on a sterile paper towel, and DNA was isolated using Mo-Bio's Plant DNA extraction kit following the manufacturer's protocol. PCR reactions were at 50 µl volume, and contained 50 mM KCl, 2.0 mM $MgCl_2$, 0.1% Triton X-100, 200 µM dNTP mix, 0.3 µM forward primer (ITS1), 0.3 µM reverse primer (ITS4) (White et al. 1990) and 1 unit Genscript Taq DNA polymerase. The PCR amplification used an initial denaturing step of 5 min at 95°C, followed by 37 cycles of 30 s at 95°C, 57 s at 57°C, and 57 s at 72°C, and a final extension step of 10 min at 72°C. The PCR product was purified using Qiaquick PCR purification kit (Qiagen), then sent to Genewiz, Inc. (Piscataway, NJ) for Sanger sequencing using primers ITS1 and ITS4. Forward and reverse sequences were aligned and edited using the program SeqTrace 0.8 (copyright 2012, Brian J. Stucky).

Sequences were searched for similarity against two different databases as follows:

(1.) **Genbank's nucleotide collection** (nr/nt) (accessed Aug 21, 2014) was searched using the 'blastn' tab in Genbank, and the 'highly similar sequences' algorithm (megablast). This was done to determine if species were likely unique to

greenbrier based on their similarity to all other reported sequences. If a sequence was found to be dissimilar from all sequences in Genbank, then it is considered more likely that the species is rare or specially adapted to greenbrier.

(2.) **A local database** was constructed using the program BioEdit v7.2.5 (copyright 1997-2013, Tom Hall). This database contained all ITS sequences from this study combined with all sequences of the UNITE database (general FASTA release version 6, 'sh_general_release_09.02.2014.', from http://unite.ut.ee/repository.php). It was searched using BioEdit's version of 'blastn' under default search parameters. This search had two purposes. First, to help assess the validity of the morphotypes by testing whether sequences of the same morphotype were most similar to each other. Secondly, the UNITE sequences in the local database were used for comparison to expert reviewed sequences ('refS' in the database), for morphotypes that were taxonomically ambiguous at higher than the species level (i.e. genus, or to confirm placement within particular species complexes). The UNITE database also contains 'repS' sequences, which represent species hypotheses generated by a computer algorithm. Since 'repS' sequences have not yet been reviewed by taxonomic experts, these were disregarded as being no more reliable than Genbank sequences.

3. Results

3.1 Pooled dataset metrics: efficiency of sampling, diversity, and colonization rate

Sample accumulation curves (Fig. 2) were non-asymptotic, indicating that at the multi-site scale stems were not exhaustively sampled. Despite this, the species richness of the epiphytic dataset was clearly higher within the level of sampling that was done, with no overlap of 95% confidence intervals. The trajectory of the curves was well differentiated showing little chance of overlap with additional sampling. Sample accumulation curves for individual sites (not shown), using each plant as a unit of sampling, also were non-asymptotic.

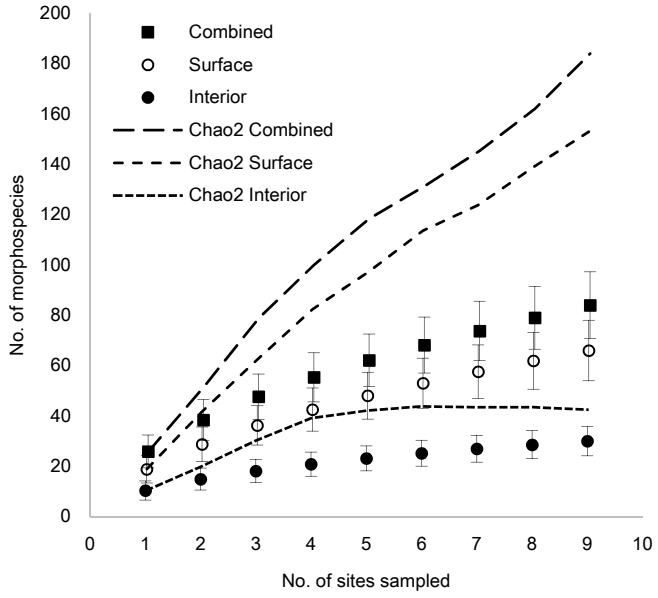

Fig. IV-2. Smoothed sampling curves, summer study

Smoothed sampling curves, and Chao 2 estimator, of greenbrier stems' culturable fungal community, using 500 bootstrap replicates without replacement; error bars show 95% confidence intervals. Each sampling unit used here (9 sites), comprised 18 x 1-cm stem samples taken from 6 stems.

Table IV-1. Colonization and morphotype richness as observed in four sample sets

Sample type	No. of samples	Samples colonized	*Total*		*Per Sample*		
			Colonies	Morphotypes	Colonies (median)	Morphotypes (median)	Max. no. of morphotypes
Stems, endophytic	162	141 (87%)	594	30	3	2	5
Stems, surface	162	150 (92%)	1,050	66	7	3	8
Leaves, endophytic	54	49 (91%)	184	13	3	2	5
Leaves, surface	54	47 (87%)	171	34	3	2	6
All combined	432	387 (90%)	1,999	80[2]			

Descriptive statistics of the four sample sets (i.e. stem surface, stem interior, leaf surface, leaf interior), pooled over all sites, are shown in Table 1. The percent colonization (i.e. percent of samples with at least one colony of any morphotype) was similar in all the datasets, at near 90%. In stems, surfaces showed higher species richness than interiors, both total and per sample. In leaves, surfaces again showed higher total species richness, but per sample richness was equivalent between surface and interior.

The ratio of epiphytic/endophytic diversity (Shannon exponential) in stems was calculated as 1.6 for the whole dataset (20.1/12.5), and 1.6 ± 0.6 averaged over the nine minor sites. For leaves, the ratio was 2.7 for the whole dataset, and 2.0 ± 1.1 averaged over minor sites.

3.2 Variation over nine minor sites: in diversity and intensity of colonization

The consistency of stem diversity metrics across each minor site can be assessed in Fig. 3. Species richness was consistently higher for epiphytes in all 9 sites. Evenness (J') was higher for endophytes in 6 sites, but was higher for epiphytes in 3 sites, though values were close between the two groups. Shannon diversity ($e^{H'}$), which incorporates both evenness and richness, was higher for epiphytes in 8 of 9 sites, with site SH3 the exception. Site SH3 also had the lowest diversity value overall (combined surface + interior dataset).

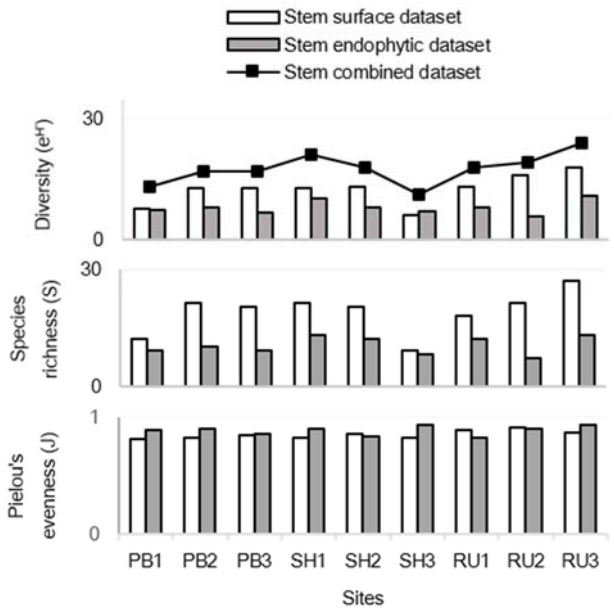

Fig. IV-3. Variation in diversity metrics over 9 sites

Diversity metrics of culturable fungal communities of greenbrier stems as they varied across the 9 minor sites. Values should not be considered absolute, as sampling curves were not asymptotic, but should be considered as trials using equivalent sampling effort at each site.

The intensity of colonization on stems varied significantly between minor sites (Fig. 4), in both endophytic and epiphytic datasets. Differences were significant even

between minor sites that were situated in the same major site. Only for the Pine Barrens locality were there no significant differences between minor sites. Epiphytes, but not endophytes showed a significant correlation between density of trees (trunk area) and colonization intensity, and the mean intensity of colonization of epiphytes and endophytes were significantly correlated to each other (see Fig. 4 caption).

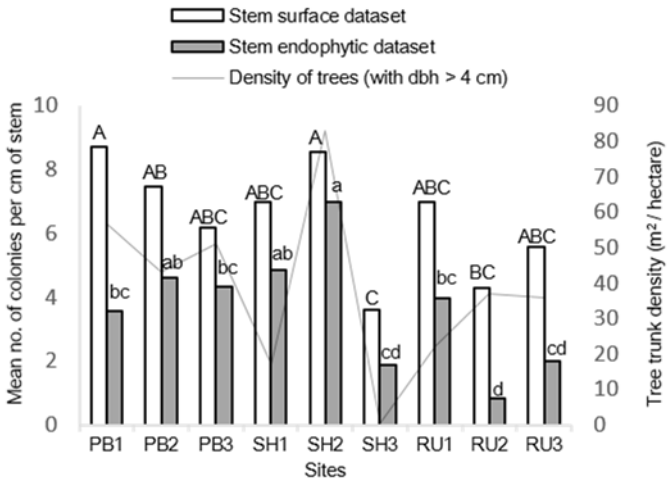

Fig. IV-4. Variation in colonization density over 9 sites

Density of colonization of culturable fungi of greenbrier stems, along with density of trees. Epiphytic colony density was significantly correlated to tree density (cor=0.67, p=0.047); endophytic colony density was not significantly correlated to tree density (cor=0.57, p=0.11); epiphytic colony density was correlated to endophytic colony density (cor=0.80, p<0.01)).

3.3 Environmental characterization: the three major sites

The compilation of climate and soil data (Table 2) supported considering the three major sites as different kinds of environments which could be characterized by a combination of soil pH and soil type, as well as differing local weather conditions in July 2011, the month preceding the August 5th sampling date. The daily minimum temperature in the Pine Barrens during that period was 6 degrees (C) colder than that of

Rutgers, and 9 degrees colder than that of Sandy Hook. And although the multi-year average daily temperature for July is the same in all three sites, in July 2011 the Pine Barrens average temperature was 4 degrees lower than at Rutgers, and 5 degrees lower than at Sandy Hook. Finally, the highest volume of precipitation, and days of precipitation, in July 2011, was in the Pine Barrens, followed by Rutgers Campus, and then Sandy Hook.

Table IV-2. Climate and landscape characteristics of major sites[a]

	Pine Barrens	Sandy Hook	Rutgers Campus
Distance between minor sites	1.0-1.3 km	0.8 - 3.8 km	0.5 - 1.3 km
Temperature (°C)			
Mean daily, July, 1981-2010	24	24	24
Mean daily, July, 2011	22	27	26
Mean daily min, July, 2011	13	22	19
Mean daily max, July, 2011	31	32	32
Precipitation (mm)			
Mean monthly total, July, 1981-2010	112	125	129
Monthly total, July, 2011	171	39	78
Days with >= .1 inch precipitation, July, 2011	8	3	5
Terrestrial characteristics[b]			
Soil type	Sand	Sand	Loam & silt loam
Soil pH	3.6-5.5	5.1-7.8	3.6-5.5
Elevation (m)	33 - 34	2 - 3	21-35
NJ climate classification	Pine Barrens	Coastal	Central
Other descriptive characteristics	Continuous forest, very little traffic	Salt aerosols, high traffic in summer, coastal storm flooding/disturbance	Urban, heavy traffic, heavily paved with discontinuous forests

[a] Details of source data given in materials and methods

[b] Soil horizon A characteristics given; reported values reflect total range over the three sites sampled in each environment

The forest composition and other vegetative characteristics in the 10 x 60 meter blocks surrounding the sampled greenbrier are shown in Table 3 (next page). The major sites Rutgers Campus and Pine Barrens both shared the presence of *Acer* and *Nyssa* trees at most sites, but otherwise were quite distinct from each other. The forest compositions for the Pine Barrens sites were the most homogeneous, dominated by *Pinus* trees. These sites could be described as pitch pine lowland forests, using Collins and Anderson's (1994) classification of New Jersey plant communities. The different minor sites within Sandy Hook and Rutgers Campus showed more variability.

3.4 The effect of locality

The NMDS and ANOSIM analysis (Fig. 5) demonstrated that epiphytic and combined datasets clustered by major site, while for endophytes the trend was similar, but with only marginally significant ANOSIM result. Combining surface and endophytic datasets led to a stronger ANOSIM result than either of the two groups alone. Clustering was clearly tightest for the Pine Barrens sites, while the other two sites showed a large spread. The NMDS ordination based on forest tree spp. similarity (Fig. 5d) reinforces the impression of Table 3, that the Pine Barrens sites shared a very similar forest composition, forming a very tight cluster compared to the other sites.

Table IV-3. Vegetative characteristics of sampling sites

						Counts of tree spp. with dbh > 4.0 cm																
Major Site	Minor Site	GPS coordinates	Site Description	Greenbrier density and habit		*Pinus* spp.**	*Acer* spp.**	*Nyssa sylvatica*	*Chamaecyparis thyoides*	*Sassafras albidum*	*Quercus* spp.	*Liquidambar styraciflua*	*Robinia pseudoacacia*	*Carya* spp.	*Fagus grandifolia*	*Carpinus caroliniana*	*Juniperus virginiana*	*Prunus serotina*	*Ilex opaca*	*Myrica pensylvanica*	*Rhus copallina*	Total
Pine Barrens; (10-13 km between minor sites)	PB1	39°52'38.8"N, 74°31'48.1"W	damp, wooded	frequent climbing		28	23	4	-	13	1	-	-	-	-	-	-	-	-	-	-	69
	PB2	39°53'4.1"N, 74°31'18.1"W	damp, wooded	frequent climbing		34	24	11	5	-	-	-	-	-	-	-	-	-	-	-	-	74
	PB3	39°53'12.4"N, 74°32'12.0"W	drier, wooded	frequent climbing		72	1	-	-	3	6	-	-	-	-	-	-	-	-	-	-	82
Sandy Hook (0.8-3.8 km between minor sites)	SH1	40°25'53.4"N, 73°59'6.0"W	dense shrubby thicket	sparse climbing		-	-	-	-	-	-	-	4	-	-	-	29	7	2	1	-	43
	SH2	40°26'18.6"N, 73°59'7.2"W	wooded	frequent climbing		-	-	-	-	-	-	-	-	-	-	-	7	8	51	-	-	66
	SH3	40°27'52.5"N, 73°59'54.7"W	damp depression, treeless	dense shrubby, monoculture		-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	2
Rutgers Campus (0.5-13 km between minor sites)	RU1	40°28'28.0"N, 74°26'14.0"W	wooded with canopy gaps	moderate shrubby		-	10	4	-	2	1	7	1	-	-	-	-	-	-	-	-	25
	RU2	40°28'46.0"N, 74°25'39.9"W	small woodlot, disturbed	sparse shrubby		-	23	2	-	-	2	4	-	5	4	-	-	1	-	-	-	41
	RU3	40°28'35.6"N, 74°25'21.4"W	swampy, wooded, large canopy opening	dense shrubby, monoculture		-	5	1	-	-	-	-	-	3	1	1	-	-	-	-	-	11

****Pinus** spp. and *Acer* spp. are almost entirely *Pinus rigida* and *Acer rubrum* with only a few exceptions

Pine Barrens shrub and sub-shrub layer dominated by spp. of *Clethra*, and Ericaceous genera *Vaccinium*, *Gaylussacia*, and *Gaultheria*

Sandy Hook shrub layer varied, SH1: dense *Myrica* and *Toxicodendrion*, SH2: predominantly trees with few shrubs, SH3: dense greenbrier monoculture, common reed *Phragmites* nearby

Rutgers shrub layer varied, RU1 *Rubus*, *Clethra*, *Toxicodendron*, RU2: *Toxicodendron*, RU3: dense greenbrier monoculture

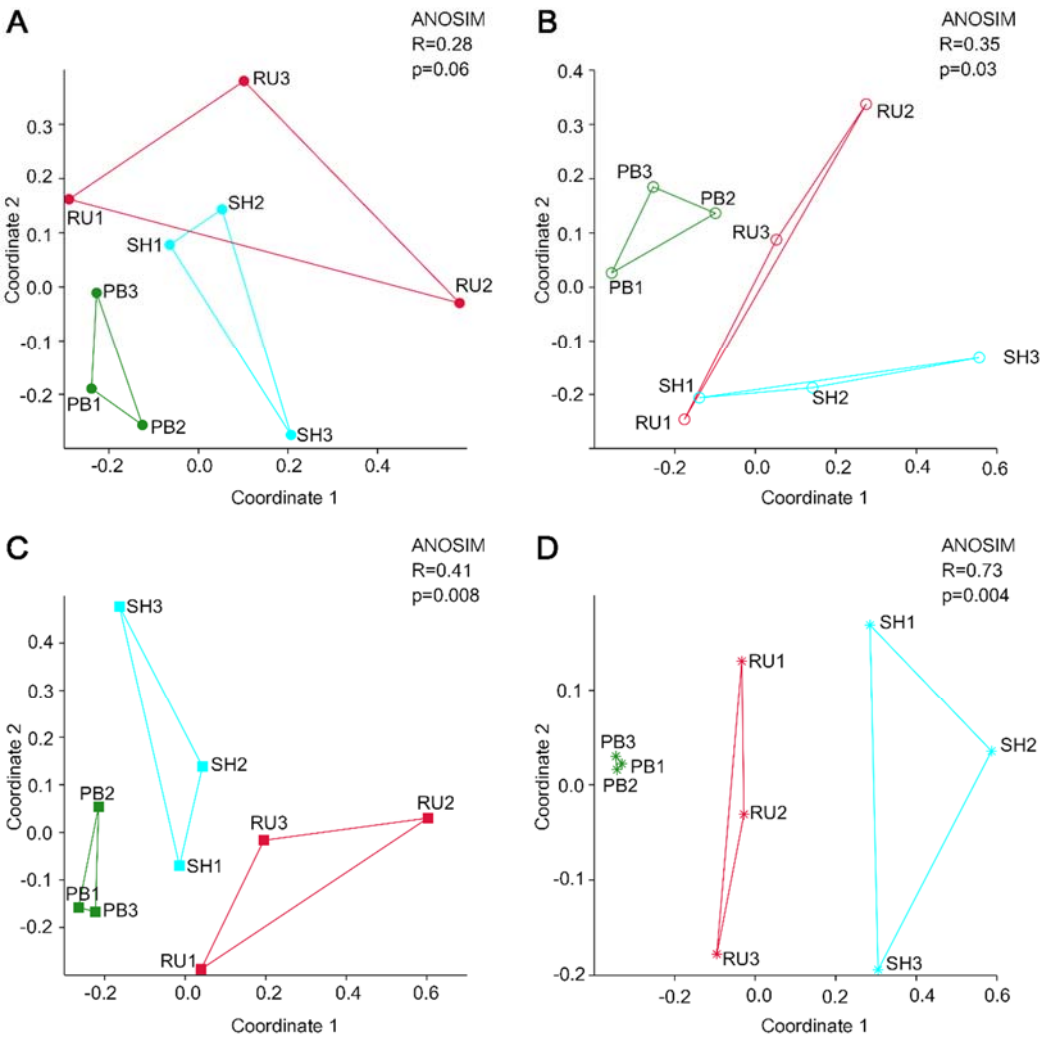

Fig. IV-5. Influence of locality on community similarity - ANOSIM and NMDS

NMDS ordinations in 2 total dimensions of greenbrier stem culturable fungal communities (A-C): (A) endophytes, (B) epiphytes, (C) combined, and (D) forest (tree) similarity. ANOSIM tests are for groupings by major sites.

From Table 4, it can be seen that the ranking of fungal species would be different depending on which major site was studied. However, the core of dominant endophytic and epiphytic species was fairly consistent. No major turnover in epiphytes was seen despite the high variation in forest type. In stems (the larger dataset), the top five endophytes, and the top nine epiphytes, were at least present at all major sites.

Table IV-4. Fungal isolates and their variation across major sites

Species / Morphotype	Frequency: All Sites	Pres. counts: All Sites	Pres. counts: PB	SH	RU
(A) Stem endophytic isolates					
Mycelia sterilia A-Yellow	36%	59	28	13	18
Phomopsis sp. 1	30%	49	16	27	6
Phyllosticta sp. 1	27%	43	8	17	18
Mycelia sterilia A-Tan	15%	25	16	4	5
Mycelia sterilia B-White[a]	14%	23	12	10	1
Colletotrichum acutatum	12%	19	0	11	8
Aureobasidium pullulans	11%	18	9	6	3
Phyllosticta sp. 2	10%	17	10	7	0
Mycelia sterilia C-White	10%	16	8	5	3
(plus 21 infrequent, total of 30)					
(B) Leaf endophytic isolates					
Phyllosticta sp. 2	54%	29	13	10	6
Phyllosticta sp. 1	48%	26	2	11	13
Mycelia sterilia B-White[a]	46%	25	14	7	4
Mycelia sterilia E-Pink[b]	13%	7	2	5	0
Phomopsis sp. 1	11%	6	2	3	1
(plus 8 infrequent, total of 13)					
(C) Stem surface isolates					
Aureobasidium pullulans	65%	105	33	43	29
Pestalotiopsis sp. 2A	47%	76	31	23	22
Pestalotiopsis sp. 2B	44%	71	37	12	22
Pestalotiopsis sp. 1	28%	45	28	6	11
Cladosporium cladosporiodes	20%	33	6	12	15
Alternaria sp.	16%	26	3	11	12
Mycelia sterilia B-White[a]	14%	22	7	12	3
Stemphylium sp.	12%	19	3	3	13
Coniothyrium-like morphotype	10%	17	1	10	6
Pestalotiopsis sp. 3	7%	12	10	2	0
Botryosphaeria spp.	6%	10	1	2	7
Penicillium sp. 1	6%	9	3	4	2
(plus 54 infrequent, total of 66)					
(D) Leaf surface isolates					
Aureobasidium pullulans	57%	31	12	8	11
Mycelia sterilia B-White[a]	35%	19	10	6	3
Dark hyphomycete 1	17%	9	6	1	2
Cladosporium cladosporioides	13%	7	0	3	4
Dark hyphomycete 2	11%	6	5	1	0
Alternaria sp.	9%	5	2	1	2
Pestalotiopsis sp. 2B	9%	5	1	0	0
Coniothyrium-like morphotype	7%	4	0	1	3

Tripospermum myrti	6%	3	0	0	1
Pestalotiopsis sp. 2A	6%	3	0	1	2
(plus 24 infrequent, total of 34)					

Two of the more obvious differences between major sites were in the rarity of Mycelia sterilia B-White (*Anthostomella* sp.) in stems at Rutgers sites, and in the absence of *C. acutatum* in all of the Pine Barrens sites.

Looking at the dominant species composition of the individual minor sites (Fig. 6), a strong recurrence of the same morphotypes is seen again across the nine sites, in both endophytic and epiphytic assemblages. It can be seen that in the Pine Barrens, the three minor sites have a remarkably consistent composition of dominant species. In Sandy Hook, sites SH1 and SH2 were very similar, while SH3, the mono-specific stand of sun exposed greenbrier, showed far less colonization and lacked the *Pestalotiopsis* component seen in the other two sites. Of the three major environments/localities, the greatest variation in minor sites was seen at Rutgers Campus sites, none of which look very similar, as reflected in the wide scatter in the NMDS. It is also striking in Fig. 6 how in epiphytic communities more of a contribution is made by the less frequently isolated species, designated as *others*.

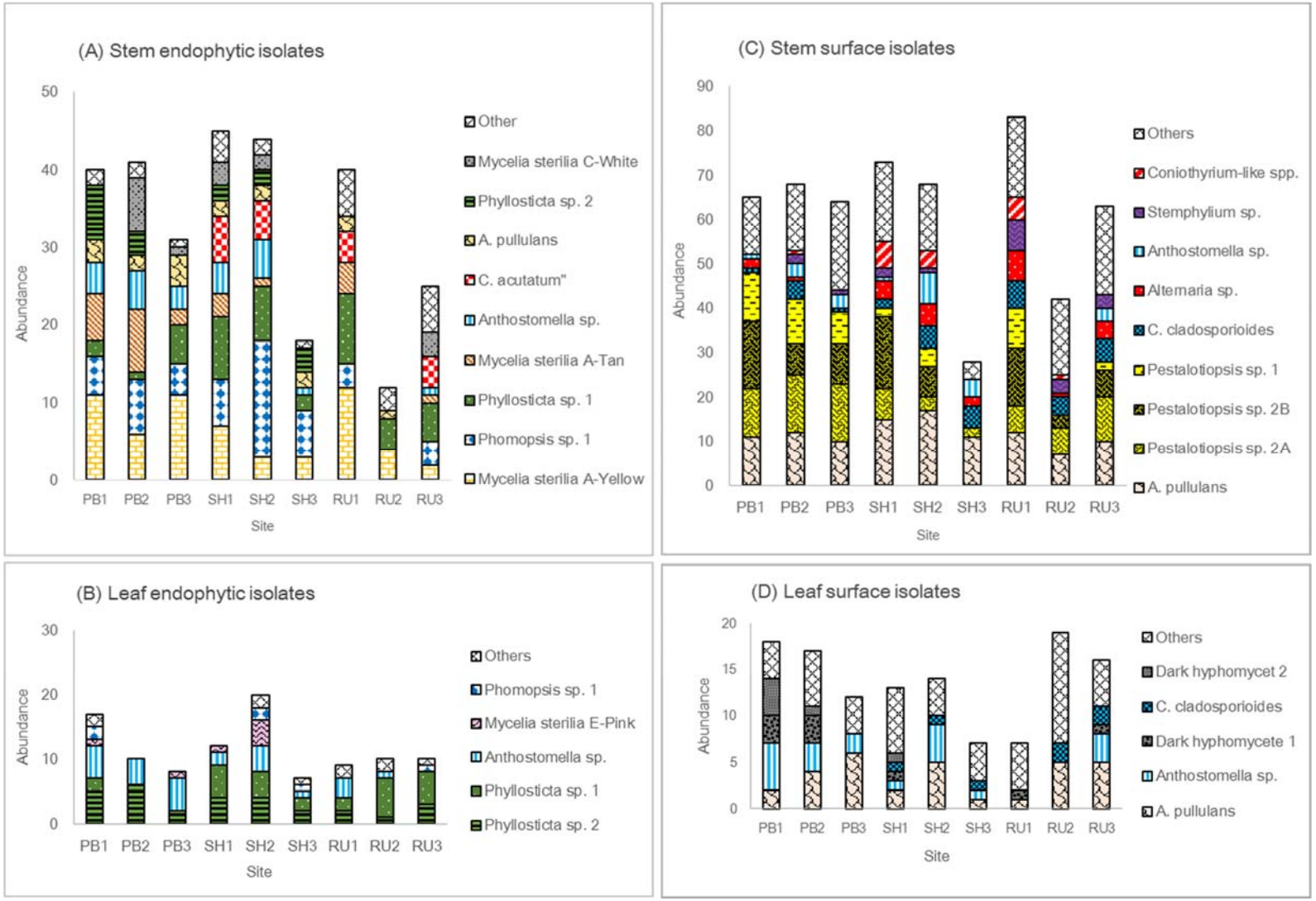

Fig. IV-6. Variation in dominant fungal species across 9 sites

Abundance is simply presence counts on the scale of 1-cm samples. Species with total frequency < 10% were lumped as 'others.'

Since the Pine Barrens minor sites had formed such a distinct cluster in the NMDS/ANOSIM analysis (Fig. 5), SIMPER analysis was performed using the combined surface/endophytic dataset to determine if any particular species or genera defined the Pine Barrens environment from the other sites. This analysis revealed that differences were caused by a great number of species without any individual very dominant contributor. However, *Pestalotiopsis* spp. (1, 2A, 2B, and 3) taken together were leading factors. All *Pestalotiopsis* spp. were more abundant in Pine Barrens sites, and together made up 25% of the difference between PB sites and other sites. Mycelia sterilia A-Yellow and Mycelia sterilia A-Tan morphotypes were also more common in the Pine Barrens, together contributing 10% to differences. Mycelia sterilia C-White (of unknown family/genus), and *Phyllosticta* sp. 2 were also more abundant in the Pine Barrens sites than in either of the other two major sites. The Pine Barrens also had lower abundance of *Alternaria* sp., *Phyllosticta* sp. 1, and *C. acutatum* than either of the other two sites. Some other differences depended on whether the Pine Barrens were compared to Sandy Hook or Rutgers (*Phomopsis* sp. 1 SH>PB>RU, *A. pullulans* SH>PB>RU).

3.5 Stem vs. leaf, surface vs. interior

ANOSIM and NMDS analysis (Fig 7) supported considering each micro-habitat as unique from each other, except with some uncertainty about leaf surface vs. stem surface fungi. The NMDS ordination showed that the separation between endophytic and epiphytic communities was largest. Secondly, endophytes of stems and leaves were fairly well separated into two clusters. Finally, despite the significant ANOSIM result, leaf surface fungi and stem surface fungi did not form distinctly separate groups in the NMDS

ordination. The leaf surface communities were very spread out. This suggests that the significant ANOSIM result for the stem vs. leaf surface comparison may partially reflect difference in the degree of dispersion in the data rather than different groups (Anderson & Walsh, 2013).

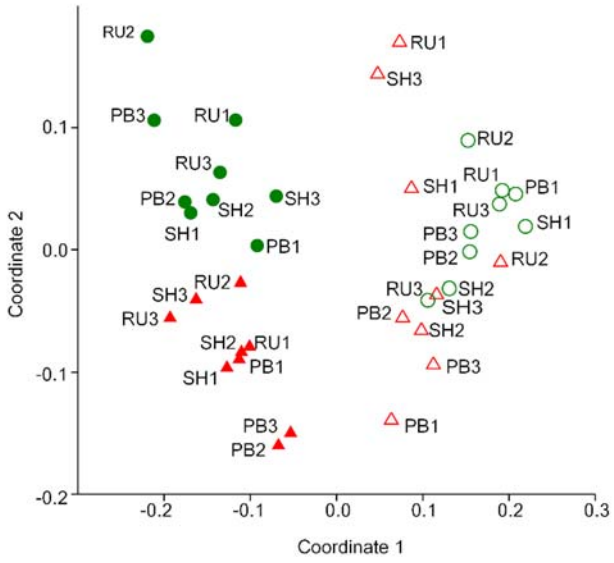

Fig. IV-7. NMDS ordination: fungal communities in four micro-habitats

NMDS ordination of 36 fungal assemblages representing 4 micro-habitats sampled at each of 9 localities (minor sites): (legend: circles = stems, triangles=leaves, open symbols=surfaces, closed symbols=interiors). ANOSIM testing showed significant differences between (1) stem: surface vs. interior (R=0.99, p=0.0021), (2) leaf: surface vs. interior (R=0.83, p=0.0001), (3) interior: stems vs. leaves (R=0.67 p=0.0001), and (4) surface: stems vs. leaves (R=0.29, p=0.0021). In this analysis, the reduced stem dataset was used to make more equitable comparisons to the smaller leaf dataset.

The strong surface vs. interior separation seen in the ANOSIM/NMDS analysis was in agreement with species lists as seen in Table 4. Most common endophytic species were rare on surfaces, and common epiphytic species rare in interiors, with the exceptions of *A. pullulans* and Mycelia sterilia B-White (*Anthostomella* sp.), which were common in both. Overlap of stem surface and stem interior community was estimated using Bray-Curtis similarity, as 0.15 for the whole site, or 0.12 ± 0.03 averaged over the

nine minor sites. For leaves, it was 0.22 for the whole dataset, 0.15 ± 0.11 averaged over the minor sites. (Sorensen QS, also useful for comparison to other studies, was 0.25 for stems, 0.26 for leaves, and similar values averaged over minor sites).

Comparing endophytic communities of leaves and stems, Table 4 shows a fair amount of overlap, but there are also clear contrasts. The endophytic community of leaves showed greater dominance in its structure compared to stems, with *Phyllosticta* sp. 1, *Phyllosticta* sp. 2, and Mycelia sterilia B-White (*Anthostomella* sp.), all showing around 50% frequency, followed by a sharp drop to near 10% frequency for the fourth and fifth ranked species. Of these three dominant leaf endophytes, *Phyllosticta* sp. 2 showed the greatest contrast between tissues, with much greater importance in the leaf community (1st ranked, 54% frequency) compared to the stem community (8th ranked, 10% frequency), and Mycelia sterilia B-White (*Anthostomella* sp.) showed a similar but slightly less pronounced leaf preference. Mycelia sterilia E-Pink (*Mycosphaerella* sp.), though subdominant, also clearly had a greater affinity for leaves (13% frequency in leaves vs. 1% in stems).

Turning to those species that preferred stems, an obvious contrast can be seen in that the sterile forms Mycelia sterilia A-Yellow and Mycelia sterilia A-Tan (suspected Microthyriaceae spp.) were very common on stems but very rare on leaves (36% and 15% of stem samples vs. 1% and 1% of leaf samples). Finally, *Phomopsis* sp. 1 clearly showed a greater presence in stems (2nd ranked, 30% frequency) compared to leaves (5th rank, 11% frequency).

Surfaces of stems and leaves had in common that *A. pullulans* was the no. 1 ranked species in both, but some clear differences also emerged. The 2nd, 3rd, and 4th ranked stem epiphytes, all *Pestalotiopsis* spp., dropped to being tied for 9th, 7th, and tied for 10th rank respectively in leaves. Mycelia sterilia B-White (*Anthostomella* sp.) became more dominant in leaf surfaces (2nd rank, 35% frequency) compared to stem surfaces (7th ranked, 14% frequency in stems), mirroring its presence as a dominant leaf endophyte. Finally, two very slow growing, dark, crust-like hyphomycetes (morphotypes: 'dark hyphomycete' no. 1 and no. 2) that were rare on stems appeared fairly frequently on leaf surfaces (Unfortunately these isolates were not at first recognized as common, or characteristic, leaf surface inhabitants, and so were not retained for sequencing). So, species lists supported that differences between surface of stems and leaves were more than just in the variability of the assemblages.

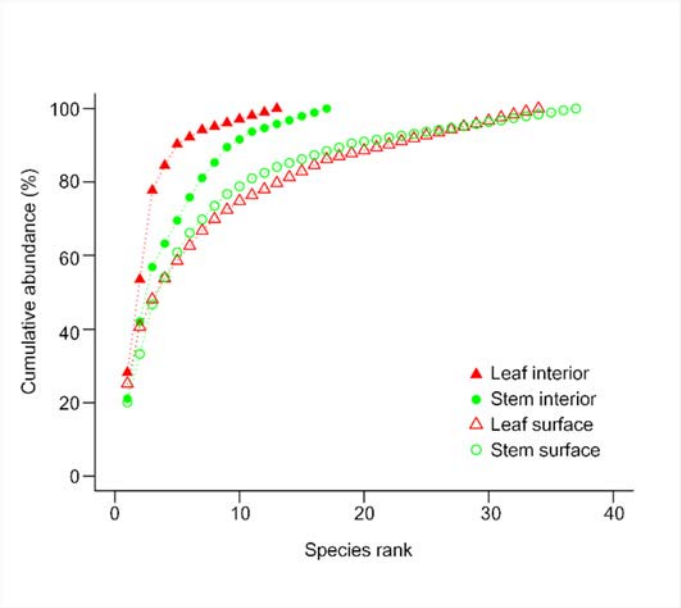

Fig. IV-8. Cumulative abundance plots: community structure in four micro-habitats

Cumulative abundance plot comparing greenbrier's culturable fungi of tissue surfaces, interiors, stems, and leaves. The reduced stem dataset was used for a more equivalent comparison to the smaller leaf dataset.

The cumulative abundance plot (Fig. 8) can be used to compare community structure between all four micro-habitats, despite some differences in tissue processing (i.e. number of tissue segments plated), since each species' abundance is considered as a proportion of total abundance. Plots that fall lower on the figure, without crossing, are considered to show a more diverse community (Magurran, 2004). Using this principal, the figure indicates that the order from least to most diverse assemblages are: leaf endophytes < stem endophytes < leaf epiphytes ≈ stem epiphytes. The figure also shows that the leaf endophyte community is strongly dominated by just three species that account for 80% of cumulative abundance. In stem endophytes it takes between 6-7 species to reach 80% cumulative abundance.

3.6 Relative influence of locality, tissue-type, and surface vs. interior habitat

The plot of average Bray-Curtis dissimilarity between small samples sets (6 x 1-cm stem segments or 6 leaf samples) (Fig. 9) showed that samples taken from surface vs. endophytic habitat showed the greatest difference, followed by those from stem vs. leaf, with geographic effects of sampling in different sites coming in last. An exception to this order was comparing samples of leaf surfaces, which were apparently highly variable from one sample to the next, with a turnover equal in magnitude to the effect of changing tissue type. Moving left to right on the figure along the dotted lines, one can see the effect of different geographic distances on species turnover. The data shows that most of the increased turnover in geographic variation is caused by small changes in location (0.5-3 km), with little gain by adding large changes in in location (40-80 km).

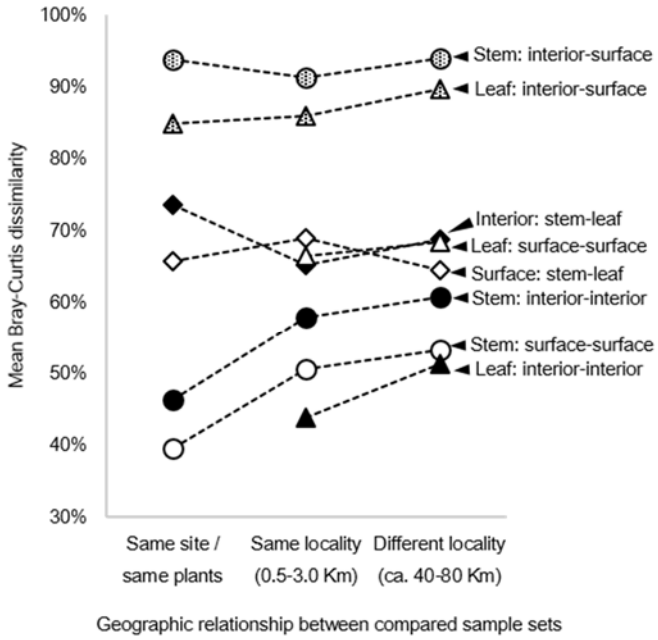

Fig. IV-9. Relative influence of locality, tissue-type, and surface vs. interior habitat

Each point represents the average of comparisons made between sample sets of 6 x 1-cm stem samples or 6 leaf samples, each of which was taken from a different plant. The number of comparisons the average is derived from varies, and standard deviation and significance is not shown; statistical significance was dealt with in the ANOSIM tests.

3.7 Seasonal comparison of summer to winter dataset (site RU1 only)

For common epiphytes, morphotypes found significantly more frequently in summer were *A. pullulans* (winter 95% CI: 4 - 11 counts, summer: 12 counts), *Pestalotiopsis* sp. 2B (winter 95% CI: 0-3 counts, summer: 13 counts), and *Alternaria* morphotypes (winter 95% CI: 0-2 counts, summer: 7 counts). Also, 7 counts of *Stemphylium* sp. were made in site RU1 in summer, while no *Stemphylium* isolates were observed in winter, so no 95% CI could be made. The only species shown to be found more often in winter was *Tripospermum myrti* (winter 95% CI: 1-8, summer: 0). However, this does not mean that it was the only epiphytic species more common in winter. As noted in the methods section, several *Phoma*-like and *Coniothyrium*-like morphotypes may also be more common in winter but were not analyzed.

For endophytes, species found with a significantly higher frequency in summer were Mycelia sterilia A-Yellow (winter 95% CI: 0-4 counts, summer: 12 counts), and *C. acutatum* (winter 95% CI: 0-3 counts, summer: 4 counts). Although it was suspected that *Colletotrichum boninense* was more common in winter, it could not be proven using the winter and summer datasets of site RU1 (winter 95% CI: 0-7 counts, summer: 0 counts).

3.8 ITS-based identification of common sterile isolates

The ITS sequence analysis is detailed in Table 5. The most dominant stem endophyte, **Mycelia sterilia A-Yellow**, was found to be distinct from the morphotype **Mycelia sterilia A-Tan**, despite their morphological similarity other than color, as reflected in the name. Both these morphotypes showed similarity in Genbank ITS hits to the sequence KPJ469652, Microthyriaceae species. As detailed in a previous paper (Zambell and White 2014), a large subunit rRNA coding sequence taken from a Mycelia sterilia A-Tan isolate also supported placement of this morphotype in Microthyriaceae (in genus *Muyocopron*).

Mycelia sterilia B-White sequences (2 sequences, different major sites) were 100% similar to each other, and 99% similar to Genbank sequence AY908990, a vouchered specimen of *Anthostomella sepelibilis,* obtained from dead stem of *Smilax* sp. (USA) (Palaez et al. 2008). Furthermore, *Anthostomella* fruiting bodies (not identified to species) were found sporulating on dead decomposing stems of greenbrier at field site RU1. These were brought to the lab, fruiting bodies placed in sterile distilled water, vortexed, diluted to 1:10 & 1:100 concentration, and plated on MEA. The colonies

formed were identical to Mycelia sterilia B-White. Together with the ITS sequence evidence, it is nearly certain that this morphotype represents an *Anthostomella* species.

Mycelia sterilia C-White could not be reliably identified, as it was most similar in ITS sequence to unidentified fungal endophytes from other plants, and its nearest refS match in the UNITE database, *Lecythophora luteoviridis* (family Coniochaetaceae), showed only 42% sequence coverage. PCR was successful for only a single isolate of this morphotype, so no comparisons could be made between sequences of the same morphotype.

Mycelia sterilia E-Pink was found to be 99% similar in 2 isolates taken from different major sites. This morphotype showed 99% similarity to Genbank sequence NR111549, type specimen of *Mycosphaerella nyssicola* - so it may be safely assumed that it represents a *Mycosphaerella* species. It was characteristic of leaf endophytes, and no other micro-habitats.

Table IV-5. Blast results for ITS sequences of isolates against local database of isolates, Genbank, and UNITE refS database

Morphotype	Local database	Genbank top matches (and UNITE refS match for ambiguous genera or species-complex)
Alternaria sp. (RU1-P5-T3-5) (2 sequences)	100% - *Alternaria* sp. (PB2-P4-T1-6)	100% - *Alternaria* sp. [KM051398]; soil, India + 2 very similar 100% - *Alternaria* sp. [KJ541477]; diseased *Nicotiana attenuata*, USA 100% - *A. brassicae* [KJ728680]; indoor air (99 perfect matches, 100% ID, 100% cov., multiple spp. names listed)
Colletotrichum acutatum (RU3-P2-N3-8) (2 sequences)	100% - *C. acutatum* (SH2-P2-N-Lf-3) 94% (78% cov.) - *C. boninense* (RU2-P3-N1-7)	100% - *Glomerella acutata* [AB443950]; *Cotinus coggygria* (smoketree), Japan 100% - *G. acutata* [AB042300] 100% (99% cov.) - Uncultured fungus [KF800117]; indoor air, USA: Missouri **refS, UNITE database** = 98% (90% cov.) *C. nymphaeae* [JQ948197]; of the *C. acutatum* species complex.
Colletotrichum boninense (RU2-P3-N1-7) (1 sequence)	≤ 94% - Other *Colletotrichum* isolates	100% - *C. gloeosporioides* [GU479899]; *Trillium tschonoskii* (per title) 100% - *C. boninense* [GU935883]; Ginseng (inferred from title) 100% - *C. gloeosporioides* [EU847425]; China **refS, UNITE database:** 99% (90% cov.) - *C. beeveri* [JQ005171]; of the *C. boninense* species complex
Pestalotiopsis sp. 1 (RU1-P4-T1-4) (4 sequences)	100% - *Pestalotiopsis* sp. 1 (PB2-P5-T2-8) 99% - *Pestalotiopsis* sp. 1 (SH1-P5-T1-7) 99% - *Pestalotiopsis* sp. 1 (PB3-P3-T3-4) ≤ 97% - other *Pestalotiopsis* (8 isolates)	99% - *P. microspora* [JX045823]; roots, *Fragaria chiloensis*, USA 98% - *Fusarium proliferatum* [EU821489]; Columbia (inferred from title) 98% - *Monochaetia camelliae* [AY682948]; pathogen, *Camellia hongkongensis*, China
Pestalotiopsis sp. 2A (RU2-P1-T-Lf-3) (3 sequences)	99% - *Pestalotiopsis* sp. 2A (PB2-P3-T3-3) 99% - *Pestalotiopsis* sp. 2A (SH2-P2-T3-1) ≤ 97% - other *Pestalotiopsis* (9 isolates)	100% - *P. cocculi* [EF055191] 100% - *P. caudata* [EF055188] 100% - Fungal endophyte [EF419946]; *Platycladus orientalis + 2 very similar*
Pestalotiopsis sp. 2B (RU1-P4-T3-3) (3 sequences)	100% - *Pestalotiopsis* sp. 2B (PB3-P2-T3-8) 100% - *Pestalotiopsis* sp. 2B (SH1-P1-T2-6) ≤ 97% - other *Pestalotiopsis* (9 isolates)	100% - *P. vismiae* [EF055221] + 1 very similar 99% - uncultured fungus [GU721793]; surface dust, USA 99% - *P. microspora* [FJ459945]; China
Pestalotiopsis sp. 3 (PB1-P2-T3-8) (2 sequences)	100% - *Pestalotiopsis* sp. 3 (SH2-P2-T1-2) ≤ 94% - other *Pestalotiopsis* (10 isolates)	100% - *P. microspora* [KJ019328]; *Elaeis guineensis* (oil palm), China 100% - *Pestalotiopsis* sp. [KF746150]; fur of sloth (*Bradypus variegatus*), Panama + 2 very similar 100% - Fungal sp. [KF467094]; *Philodendron guttiferum*, Ecuador (48 perfect matches, 100% ID, 100% cov., multiple spp. names listed)
Phomopsis sp. 1 (RU1-P6-N2-2) (3 sequences)	99% - *Phomopsis* sp. 1 (SH2-P4-N-Lf-2) 99% - *Phomopsis* sp. 1 (PB2-P6-N2-9) 94% - *Phomopsis* sp. 2 (RU2-P1-T-Lf-1)	97% (98% cov.) - *Diaporthe detrusa* [KC343062]; *Berberis vulgaris*, Sweden 97% (98% cov.) - *Diaporthe detrusa* [KC343061]; *Berberis vulgaris*, Austria 97% (99% cov.) - *Diaporthe neoviticola* [KC145831]; *Vitus vinifera*, Australia
Phomopsis sp. 2 (RU2-P1-T-Lf-1) (1 sequence)	≤ 94% - other *Phomopsis* (3 isolates)	99% (99% cov.) - *Phomopsis* sp. [EF432292]; *Brassica nigra*, USA 99% (98% cov.) - *Phomopsis* sp. [HQ171093]; peach, China 99% (99% cov.) - Uncultured fungus [KF742587]; asymptomatic, live *Viburnum* sp., USA

Phyllosticta sp. 1 (RU1-P2-N2-7) (2 sequences)	100% - *Phyllosticta* sp. 1 (PB1-P3-n-Lf-3) ≤ 93% - other *Phyllosticta* (3 isolates)	95% - *P. cryptomeriae* [AB454294]; leaf, *Cryptomeria japonica*, Japan + 2 very similar 95% - *G. philoprina* [FJ824768]; *Taxus baccata*, Netherlands 94% - *Guignardia* sp. [AB454287]; leaf, *Podocarpus macrophyllus*, Japan
Phyllosticta sp. 2 (RU1-P2-N-Lf-1) (3 sequences)	100% - *Phyllosticta* sp. 2 (SH1-P2-N2-6) 100% - *Phyllosticta* sp. 2 (PB1-P5-N3-6) ≤ 93% - other *Phyllosticta* (2 isolates)	99% - *Phyllosticta* sp. [AB454329]; leaf, *Gaultheria shallon*, Japan 99% (99% cov.) - Fungal sp. [KC867797]; *Rhododendron occidentale*, USA 99% (99% cov.) - *Phyllosticta* sp. [DQ377928]; *Eucalyptus globulus*, Spain
Mycelia sterilia A-Tan (RU3-P2-N3-4) (2 sequences)	99% - Mycelia sterilia A-Tan (50A-N-1t-12, study1) 94% - Mycelia sterilia A-Tan (PB1-P5-N2-2) ≤ 93% (67% cov.) - Mycelia sterilia A-Yellow (2 isolates)	88% (64% cov.) - Dothideomycetes sp. [JQ760089]; endophyte, *Serenoa repens*, USA: Florida + 5 very similar 89% (60% cov.) - Fungal sp. [JX014394] leaf, *Illicium verum* + 2 very similar 89% (60% cov.) - Fungal sp. [GU985230]; *Hyperzia serrata*, China: Jiangxi province 89% (61% cov.) - Dothideomycetes sp. [JQ760271] - lichen thallus, *Cladonia leporina*, USA: Florida 89% (60% cov.) - *Microthyriaceae* sp. [KJ469652] - *Paspalum conjugatum*, Panama **refS, UNITE database**: no significant match
Mycelia sterilia A-Yellow (RU1-P5-N2-3) (2 sequences)	99% - Mycelia sterilia A-Yellow (SH2-P2-N1-2) ≤ 93% (58% cov.) - Mycelia sterilia A-Tan isolates	93% (50% cov.) - *Microthyriaceae* sp. [KJ469652]; *Paspalum conjugatum*, Panama 93% (50% cov.) Fungal endophyte [KF435946]; endophyte, leaf, *Psychotria* sp., Panama 90% (55% cov.) Uncultured *Mycoleptodiscus* clone [KF718238]; rhizome, *Alpinia officinarum* **refS, UNITE database**: no significant match
Mycelia sterilia B-White (PB1-P3-N2-4) (2 sequences)	100% - Mycelia sterilia B-White (SH2-P5-T2-7)	99% (98% cov.) - Sordariomycetes sp. [JQ761985]; endophyte, *Tsuga canadensis*, USA: Florida 99% (98% cov.) - Sordariomycetes sp. [JQ761984]; endophyte, *Tsuga canadensis*, USA: North Carolina 99% (98% cov.) - Sordariomycetes sp. [JQ761898] endolichenic, *Pseudevernia consocians*, USA: North Carolina 99% (90% cov.) - *Anthostomella sepelibilis* [AY908990] **refS, UNITE database**: no significant match
Mycelia sterilia C (RU3-P3-N1-1) (single sequence)		88% (97% cov.) - Fungal endophyte [EU686971]; *Streptochaeta spicata* 88% (88% cov.) - Fungal endophyte [KF435931]; leaf, *Psychotria* sp., Panama 88% (88% cov.) -Fungal endophyte [KF435425]; leaf, *Costus laevis*, Panama **refS, Unite database:** 95% (42% cov.) - *Lecythophora luteoviridis* [HE610333]
Mycelia sterilia E (PB1-P4-N-Lf-1) (2 sequences)	99% - Mycelia sterilia E (SH2-P4-T-Lf-1)	99% (99% cov.) - *Mycosphaerella nyssicola* [HQ162263]; Leaves, *Nyssa* sp., USA: Maryland + 1 very similar 99% (98% cov.) - Dothideomycetes sp. [JQ761674]; endophyte, *Kalmia latifolia*, USA: North Carolina 99% (97% cov.) - *Mycosphaerella nyssicola* [NR 111549] Type material; *Nyssa* sp., USA: Maryland **refS, Unite database:** 96% (82% cov.) *Ramularia cynarae* [HQ728117]

3.9 ITS sequence similarities

The ITS sequence data (Table 5) supported the morphotype groupings of dominant species that were previously made and supported splitting similar morphotypes in instances that were difficult to call (e.g. *Pestalotiopsis* sp. 2A vs. 2B, and Mycelia sterilia A-Tan vs. Yellow) rather than lumping. The UNITE database was useful for confirming that isolates of *C. acutatum*, and *C. boninense* from this study did show high similarity to isolates of the *C. acutatum* and *C. boninense* species complexes. The terms *C. acutatum* and *C. boninense* are more akin to a genus name in the sense that they contain multiple species (Damm, et al., 2012; Damm, et al., 2012).

Sequences could roughly be divided into 2 groups:

(1) Sequences that were more similar to their own morphotype in this study than to any other sequences in Genbank. This suggests that these morphotypes are undescribed, at least within any recent study that included molecular data. These were ***Phomopsis* sp. 1** (3 isolates, 3 major sites), ***Phyllosticta* sp. 1** (2 isolates, 2 major sites), ***Phyllosticta* sp. 2** (3 sequences, 3 major sites), **Mycelia sterilia A-Yellow** (2 isolates, 2 major sites), **Mycelia sterilia A-Tan** (3 isolates, 2 major sites), and **Mycelia sterilia B** (2 sequences, 2 major sites). **Mycelia sterilia C** only had a single sequence available, but its nearest match was 88% similar in Genbank, also suggesting it is undescribed in modern studies.

(2) Morphotypes that were equally related to each other as to Genbank sequences. Whether they are novel isolates depends on how species specific the ITS gene is within

these genera. These were ***Alternaria* sp.** (2 sequences, 2 major sites**), *Colletotrichum acutatum*** (2 sequences, 2 major sites), and **Mycelia sterilia E-Pink** (2 sequences, 2 major sites). The designation of four ***Pestalotiopsis*** morphotypes in the present study also was supported by the ITS data, in that these showed 99-100% similarity to their own morphotype, and 97% or less to any others. However, Genbank results showed 100% similarity to sometimes multiple different species names, demonstrating that ITS will not be sufficient to determine if these are new or previously described species.

4. Discussion

4.1 Influence of geographic locality on phyllosphere communities

The hypothesis that the surface community would be more subject to the influence of locality than the endophytic community was supported, in that ANOSIM was significant for the surface ($p=0.03$), but not interior ($p=0.06$). However, considering the higher R and p-value ($p=0.008$) of the combined dataset, it can be inferred that both groups contribute to a small, but significant effect of locality. Since there was a larger contingent of less common species in the epiphytic community, these may have contributed to distinguishing local regions, and producing a lower p-value in epiphytes. Specifically, endophytes had 9 species of ≥10% frequency, and 21 less common. The epiphytes also had 9 species at ≥10% frequency, but had 57 less common species.

The results of the geographic portion of the study, however, must be analyzed with an eye for not only significance of effect, but magnitude. Compared to tissue-type, surface vs. interior, or season, the effect of locality was subtle for both epiphytes and endophytes. In terms of dominant species, the hypothesis that the epiphytic community

would be determined by the surrounding vegetative (forest) community was largely refuted. I expected that there might be a different core group of dominants in each environment. To the contrary, a core group of *A. pullulans*, followed by three *Pestalotiopsis* spp. appeared on stems in each major site and most minor sites.

It is not surprising to find *A. pullulans*, *C. cladosporioides* and some other hyphomycetes at all or most sites, as these are well known to be ubiquitous. The presence of the same three *Pestalotiopsis* spp., however, often in similar proportions (e.g. compare site PB1 to site RU1), is more interesting, considering that *Pestalotiopsis* is a species rich genus. The presence of the same three species on greenbrier stems at distant and dissimilar sites suggests strong host selectivity for the poorly understood coelomycete component of the epiphytic phyllosphere, rather than spillover of dominants from the surrounding forest. The single gene ITS data supports that these three morphotypes are distinct from each other, but consistent within each single morphotype, within 99% similarity. The sharp drop in rank and frequency of *Pestalotiopsis* spp. in leaf surfaces vs. stem surfaces (Table 4) further emphasizes the selective nature of the plant surface substrate. While other studies have concluded that *Pestalotiopsis* is not host specific, there is a poor understanding of how the ecological niches *Pestalotiopsis* spp. differ.

Endophytes, like epiphytes, were found to be largely consistent between major sites. Rollinger and Langenheim (1993), similarly, found the same four dominant endophytes in needles of *Sequoia sempervirens* (coastal redwood) at most sites along a latitudinal gradient on the U.S. West Coast. Verma et al. (2007), in a multi-site study, also concluded that core endophytes remained the same in different locations. However,

just as I found that the average number colonies per cm of stem varied significantly between minor sites, previous studies have found significant variation in similar measures of colonization density ('relative isolation frequency') either by all species pooled (Santamaria and Diez 2005), or by certain dominant species (Gore and Bucak 2007). In terms of relative influence of different factors, Wang and Guo (2007), similarly, found that in *Pinus tabulaeformis* endophytes, tissue-type, and age of tissue, was far more important than location, and that while isolation frequency varied, species composition was not largely affected by location. In a study of phyllosphere fungi of *Pinus* spp. across six plantations in northern Spain, Zamora et al. (2008) showed that isolation method (sterilized, unsterilized, moist chamber) and season were factors that caused clustering in an ordination, not location.

The more geographically variable of the stem endophytes were *Colletotrichum acutatum*, *Anthostomella* sp., and *Phyllosticta* sp. 2. As the last two of these were also species that tended to dominate leaves, perhaps adaptation to leaves leads to a more inconsistent presence in stems. Species within the *C. acutatum* species complex are often host generalists, so that its presence may be influenced by the presence of other hosts in the surrounding forest.

Studies that have examined geographic variation in epiphytes are rare. Santamaria and Bayman (Santamaria and Bayman 2005) found a rather more substantial variation in dominant epiphytic genera (*Pestalotiopsis* and *Botryosphaeria*) in a study of coffee (*Coffea arabica*) trees across five sites in Puerto Rico (it is unclear how many species these genera represent). However, this variation was not specific to epiphytes as they also

found great variation in endophytic communities as well. Perhaps this variation is reflective of the tropical climate in that study. Photita et al. (Photita et al. 2001) studied endophytes of understory banana (*Musa acuminata*) plants at different wild sites in Thailand, finding that while some species were fairly consistent (e.g. *Colletotrichum musae*, *Colletotrichum gloeosporioides*, *Guignardia cocoicola*, and some Mycelia sterilia), at least appearing in all sites, others were wildly variable. For example, *Deigtonella torulosa* was in 65% of leaf tissue segments at site I, 1.3% at site II, and absent from sites III-V in that study. Another species, *Cordana musae,* was very dominant at site V, being present in 63% of leaf tissue segments, but only present in 3.3% of segments at site I, 2% at site III, and absent from two other sites. This is the type of turnover I expected to see in epiphytic dominant spp. but did not. Thus it seems that a consistent core of endophytes and epiphytes, that is, a lack of major turnover in dominant species, is characteristic to non-tropical climates.

Another study of epiphytes, Pereira et al. (2002), examined epiphyllous fungi in two micro-environments, a northern and southern slope in an arid Mediterranean ecosystem, that were locally near to each other. They found high similarity with the same four dominant taxa making up 80 and 85% of the isolates from each of the two sites. The five plants sampled in that study showed some host preference of certain species, one fairly common mycelia sterilia morphotype, and relatively rare occurrence of coelomycetes. Perhaps the very low frequency of coelomycetes was due to either the environment, or the sampling method of plating water in which the leaf had been agitated for 60 minutes after an initial prewash. Methods that involve plating of tape, as in the present study, or plating of washed tissue, consistently turn up coelomycetes in higher

frequency. The use of multiple methods in epiphytic sampling (e.g. culture-dependent and independent, plating of wash waster, plating of tape) would probably be informative in future investigations of the epiphytic mycoflora.

The clustering effect of minor sites within the same environment may have been caused by a number of difficult to disentangle aspects of the major sites, including dispersal between close physical proximities, shared local weather variation, shared atmospheric conditions (e.g. salt aerosols, pollution), genetic similarity of greenbrier in nearby proximities, similar soils that may affect hydrology and decomposition of litter, and shared plant community at the larger landscape scale. Beyond the immediate 10 x 60 m sampling sites, the Pine Barrens and Sandy Hook have distinct plant flora over many kilometers that might influence the fungal community observed on greenbrier (Chrysler 1930; Harshberger 1916). The area around Rutgers campus, a heterogeneous mix of urban development, along with both wild and gardened land, is difficult to generally characterize, but obviously different from the general flora of Pine Barrens and Sandy Hook. However, the pattern of clustering, in which the Pine Barrens clustered much more tightly than the other two environments, suggests that the plant community and forest structure in the immediate vicinity of sampling was more important than the general plant flora at the multi-kilometer scale. The Pine Barrens minor sites, unlike the other environments, were much more homogeneous in the immediately surrounding trees (Table 3, Fig.5d), had similar density of trees (Fig. 4), and were probably of a similar age as tree trunk widths were similar.

It is surprising that the Sandy Hook community did not show a more distinctly homogeneous fungal community due to salt aerosols assumed to be present at the site. For example, Brown and Di Meo (1972) studied the epiphytic lichen *Parmelia perlata* on oak trees on a peninsula in South Devon, England, demonstrating that samples taken closer to the sea showed lower abundance of the lichen, along with higher chloride content in the lichen thallus. Another lichen in the same study, *Parmelia caperata,* on the other hand, showed a steady chloride content and relatively steady abundance no matter the distance from the coast. While my results show nothing particularly distinct about the Sandy Hook environment, future studies of the coastal environment should employ other sampling techniques that might capture very slow growing or culture independent species.

Two peculiar sites, in that they were sparsely colonized, were sites SH3 and RU2. Site SH3 also had the densest biomass of greenbrier, with much standing dead material, showing that the presence of dense greenbrier does not ensure high fungal colonization. The low colonization at this site may be related to the lack of canopy there, leading to intense UV exposure, and shorter periods of moisture. It was also observed during sampling that plants taken from this site had thicker, more leathery leaves, and were a slightly different color than those from other sites, suggesting adaptation to the high UV exposure. Site RU2, however, also low in colonization, and lacking many endophytic dominants, was not lacking canopy. It did have in common with site SH3 that, unlike all other sites, it was near a paved road (SH3 was bordering a paved bike trail, with a road also nearby). Both these sites are probably more likely to experience splashing or runoff of salt applied in winter, or possibly air pollution.

4.2 Influence of tissue type

My finding of clear differences in endophytic community of green leaves vs. stems is in contradiction to Persoh's (2013) finding that green stems and leaves of European mistletoe (*Viscum album*) did not differ in endophytic composition. However, it is in agreement with studies of other monocots, such as Photita et al.'s (2001) study of wild banana (*Musa acuminata*) and Bussaban et al.'s (2001) study of wild ginger (*Amomum siamense*), both of which showed that endophytic communities of leaves differed from those of the green pseudostems of those species.

In the previous winter study of greenbrier (Zambell and White 2014) I found that different heights along the stem favored different endophytes, which suggests that there are internal differences in the stem at the scale of 10's of centimeters. I reasoned that differential success in colonization or survival of the different environments might lead to the perceived preference. The same reasoning could be applied to the difference observed between leaf and stems. On the other hand, major differences in the surface of stem vs. leaf tissue may allow for selective adaptations at the dispersal phase to avoid adhesion and germination of spores in a poorly suited environment, in which case, the term 'preference' would be more appropriate. Species that show preference for a particular tissue may rely on chemical cues for attachment or germination (Petrini 1996), or stems may be preferentially selected for by winter or early spring dispersal before leaves have emerged.

There are multiple reasons that certain species may be adapted to avoid certain tissues. Carroll and Petrini (1983), noting the distribution of different fungi that preferred basal (petiole) versus other sections of *Pinus* needles, found evidence of substrate adaptation in the digestive enzymes produced. Those they classified as 'petiole fungi,' were typically able to utilize cellulose, pectin and other hemicelluoses, while 'blade fungi' (those with no preference for the petiole) typically could only digest pectin. Similar substrate adaptation may also play a role in adaptation to stem vs. leaves in greenbrier.

Leaves also have a different life-span than stems, and upon senescence move into different fields of competitive and abiotic conditions. Leaves fall off throughout the autumn and winter, while stems are perennial, and remain standing after death rather than immediately entering the moist litter layers. Species well adapted to the stem life-span, if they accidentally colonize leaves, may have insufficient time to develop on leaves, might fail to compete in the litter, or might fail to capitalize on a long, multi-season reproductive period.

Indirect evidence was found in this study showing that some endophytes might exhibit a long, multi-seasonal life cycle, persist in standing dead stems long after death of the tissue, particularly for Mycelia sterilia species. As noted in the results, *Anthostomella* ascospores from well decayed, though still standing, greenbrier stems produced colonies identical to Mycelia sterilia B-White, a morphotype proven to be an *Anthostomella* sp. by ITS sequence. The sequences of Mycelia sterilia A-Tan and Mycelia sterilia A-Yellow, showing affinity for family Microthyriaceae, also suggest a link between these and *Muyocopron* fruiting bodies frequently observed by ourselves and others (Luttrell 1944)

on dead stems of greenbrier. Also, although not an example of long term persistence, in dead tips of still living stems, I frequently observed fruiting bodies producing spores of one or more *Phomopsis* species, which may be the same as the endophytic *Phomopsis* spp. cultured.

The tissue type was also significant for epiphytic communities. This is in agreement with past findings that the life-span or resource uptake strategy of leaves selects for different epiphytic communities (Flessa et al. 2012; Kembel and Mueller 2014), as stems and leaves have different life-spans, probably different nutrient resource strategies. The surfaces also are different physically as a microenvironment. Microscope observations show a very different texture, as the interlocking epidermal cells of the stem, upraised in the center, create a distinct pattern of hills and valleys at the microbial scale.

4.3 Seasonality of phyllosphere species

The phenomenon of seasonal turnover was supported by my results, in agreement with other studies of endophytes, epiphytes or mixed phyllosphere species [e.g. *endophytes*—Tadych et al. 2012; Mishra, et al., 2012; Unterseher, et al., 2007; *epiphytes*—Breeze and Dix 1981; Mishra and Dickinson 1981; *mixed phyllosphere*—Thomas and Shattock 1986; Jumpponen and Jones 2010).] Some seasonal changes observed in this study were major shifts. For example, Mycelia sterilia A-Yellow, the most common endophyte in this study, was rare in winter. The *C. boninense* morphotype also probably underwent a major shift to become rare in summer, although I did not prove this statistically in my analysis which only included site RU1. It dropped from 35%

frequency in winter 2010 (or 19% in the modified dataset), to 1% (2 counts) frequency across all nine sites in the present study. While the absence of this morphotype at site RU1 fell within the broad 95% CI of the winter results, it seems unlikely that it would be so infrequent across all nine sites in summer unless there was some seasonal effect.

In both winter and summer studies, sample number was similar (160 vs. 162 1-cm samples), and the epiphytic species richness was similar (65 vs. 66 morphotypes) and still climbing. However, there was a strikingly lower endophytic species richness in the present study (51 vs. 30 morphotypes), and the Chao 2 estimator suggested that it would not climb much further. The higher endophytic richness in the winter study may be due to cooler, wetter conditions of late winter/early spring vs. the hot dry conditions of early August. However, it may be that stems sampled in March had all been in the field for at least one growing season and were older on average than those sampled in summer, many of which may have just grown up in the preceding spring; stems extend in length quickly early in the growing season and strengthen over time.

The major changes observed between seasons suggest that seasonality should be more important than locality. Contradicting this, several authors have concluded that location may be more important than season in determining endophytic communities, including Collado et al.'s (1999) study of *Quercus ilex* in four locations in central Spain, and Gore and Bucak's (2007) study of *Laurus nobilis* in Western Anatolia, Turkey. These conclusions, however, may be explained in that climatic conditions varied more strongly in these studies between different sites. Gore and Bucak (2007) sampled sites with wide variation in elevation and rainfall, and found that two sites that shared similar rainfall and

were near to each other were most similar. Collado et al. (1999) found subtle differences, wherein the importance of season seemed to vary with different sites, and whether they were hilly and sheltered, or exposed. My sites showed some microclimate differences, but no major difference in rainfall, elevation or temperature. At the same time, there is a strong seasonal change between March and August in the Mid-Atlantic region of the U.S., perhaps explaining why season should be more important in my study. For example, the state-wide mean temperature is 40.1°F (4.5°C) in March, versus 72.5°F (22.5°C) in August (per website of the state climatologist, data from 1895 to 2015: http://climate.rutgers.edu/stateclim_v1/data/index.html; accessed March 1 2015).

4.4 Epiphytic vs. endophytic diversity

Our measurement of about double the species richness from leaf interior tissues to surfaces (Table 1) is in agreement with observations of leaves in temperate zone studies (Osono and Mori 2004; Osono 2014). It was also found that greenbrier stem surfaces host a more diverse culturable fungal community than interiors, in agreement with the more limited, single site, winter dataset (Zambell and White 2014). This is supported by the overall species accumulation curve, the finding that species richness was greater in all nine minor sites, and diversity as Shannon exponential was greater in 8 of 9 minor sites. The one site that differed, site SH3, was lacking in canopy. Perhaps high UV exposure is a stress to which some of the epiphytic species are not well adapted (Pugh and Buckley 1971; Unterseher 2007), causing reduced surface diversity.

4.5 Conclusions

An important conclusion from this study is that surface communities are similar in their geographic variation to that of endophytic communities, and surface fungi are apparently characteristic to the plant studied. The idea that the surface community is a passive representation of the general environment was not supported by the evidence. The surface of each tissue, and each species of plant probably represents a unique habitat that will attract a characteristic fungal community. In the future, multi-gene phylogenies will be needed to place the endophytes and epiphytes found in this study into broader context, establish their identity as new or already described species.

Chapter V. A framework for understanding endophytic community assembly and structure: unanswered questions & future strategies

Abstract

Endophytes of aerial plant tissues have been studied for a variety of reasons, often focusing on biodiversity, potential mutualistic benefits to plants, and the search for useful fungal metabolites. However, we lack a basic framework for studying how these communities are assembled, probably because researchers are hesitant to generalize about the life-cycles of endophytes. In this chapter, as a starting point, I discuss what endophytes are, and what can be generalized about them. I emphasize what are probably the most common endophytes of aerial plant tissues, those that are horizontally transmitted and form localized infections—Rodriguez et al.'s (2009) Class III endophytes. I emphasize moving beyond snapshots of already formed endophytic communities, to experimental approaches to quantifying dynamics of colonization and survival of colonies. I also discuss the need to disentangle niche requirements from competitive outcomes. The exploration of patterns is also discussed, including the use of species abundance plots to explore trends, and the use of phylogenetic analysis to gain an evolutionary-ecological perspective. Finally, I review what is known about plant secondary metabolites in structuring endophytic communities, and how this area is one of the most promising unexplored areas of endophytology. While my emphasis is on endophytes, the ecology of surface organisms is also discussed. Ultimately, the field of surface and endophytic fungi should be merged to a more whole phyllosphere understanding. Throughout, I have tried to identify key unanswered questions that should be tractable to answer in the future with well-chosen study systems.

1. Introduction: Endophytes and the phyllosphere community

The living tissues of terrestrial plants are both habitat and potential food source to a variety of microbes. The aerial portions of plants, though exposed to UV and lacking the moisture of the soil, are no exception. The term "phyllosphere" is sometimes used to describe the microbial habitat that is the surfaces of leaves (alternately termed the "phylloplane"), or plant surfaces in general—I define it here as all living parts of the plant that are found above ground that can be used as a microbial environment. Research into fungal endophytes of various trees has suggested that biodiversity of fungi associated

with aerial plant parts may be tremendous, including both generalist and specialist species. The structure and taxonomic make-up of of these communities varies in different plants and different latitudes (Arnold et al. 2001; Arnold et al. 2007). Considering the vast variety of physical, chemical, biological defense properties found in plants, this is not completely unexpected. On the other hand, one could imagine the exposed surfaces of living plants to be so turbulent and inhospitable that only a few generalists would dominate. Recent evidence indicates, however, that although many generalist species are known to colonize plant surfaces, biodiversity of fungi here too may be vast and go well beyond a few ubiquitous phyllosphere inhabitants (Lindow and Brandl 2003, Kembel and Mueller 2014, Chapter III and IV).

The desire to quantify, and in some cases describe, the fungal biodiversity found associated with plants has been a driving force in the study of endophytes. Other motivations include the search for novel secondary metabolites of medical or industrial interest, and the search for biological agents that might positively affect crops or ornamentals, or negatively affect competitor weeds. All of these goals might be better served by taking a step back to ask: (1) what is our basic understanding of the phyllosphere ecology, (2) what are the major holes in that framework, and (3) how can these be addressed? Mycological ecology of the phyllosphere, or more generally, microbial community ecology of the phyllosphere, is an area in which there is still a need for both basic exploration and, perhaps more so, the building of a conceptual framework. Difficulties of the field include a vast number of un-described species, difficult-to-demarcate boundaries of individual biological entities, and often a lack of basic life-history knowledge. Furthermore, the identification of visible patterns available to the

macroscopic ecologist, are not available since most hyphae are indistinguishable. These difficulties, and the resulting mystery, are also part of the allure of the field.

The phyllosphere habitat

The phyllosphere has been utilized as a substrate by filamentous fungi at least since the Devonian period, ca. 400 million year ago—fossilized remains show that the upright axes of the primitive plant *Asteroxylon mackiei* were colonized by the ascomycete *Paleopyrenomycites devonicus* (Taylor et al. 2005). Putative endophytic remains in aerial organs as early as the Carboniferous have been described (Krings et al. 2012). Fossils of leaf surface fungi are also common from the *Cretaceous* period onward (Taylor & Osborn, 1996), implying a long association of filamentous fungi with the aerial organs of modern groups of terrestrial plants as they diversified. This probably has resulted in important consequences for plant biology.

Microbial colonists of both plant surfaces and interiors probably follows similar pathways of dispersal and adherence. Later, niche specialization may come into play as species that are primarily epiphytic must survive the plant surface environment, characterized by stresses of UV exposure, frequent desiccation, wind, vibrations, and antimicrobial compounds (Juniper 1991). Endophytes, on the other hand, must in some way infiltrate the surface and survive the internal environment, which is probably well sheltered from UV exposure, more humid than the surface, less variable in temperature, but more variable in CO2 concentration (Juniper 1991). The chemical environment of the interior is also most likely different from surface chemistry, lacking secreting trichomes and waxy surface layer—some compounds may be at higher concentration while others

are at lower. Unlike epiphytic fungi, endophytic fungi may enjoy protection from UV-B via the plant's own defense systems, including phenolic compounds, polyamines, waxes, and alkaloids that may absorb UV rays or scavenge the reactive oxygen species (ROS) that result from UV radiation. On the other hand, endophytes might also be exposed to ROS that is generated inside plant tissues internally as a byproduct of photosynthesis, particularly at extreme high or low temperatures (Lambers et al. 2008). Reactive oxygen species are a hazard to plants and fungi as they can damage biological membranes, DNA and other cellular structures (Lambers et al. 2008).

Endophytes and epiphytes are distinct, but slightly overlapping communities

The study of surface fungi has typically fallen into the broad category of 'phyllosphere' research, which traditionally has emphasized the variety of organisms (bacteria, yeasts, fungi, algae) on the leaf surfaces using a variaty of sampling techniques to study different groups, has emphasized dynamics of certain dominant species rather than general biodiversity, and has more often focused on agricultural systems. Endophytology, by contrast, is characterized more by sampling in natural environments, a focus on filamentous fungi isolated from surface sterilized plant segments on nutrient media, the application of statistical/community ecology analysis, and has often emphasized biodiversity and mycoflora of specific plant species.

There are studies, however, in which both surface and endophytic fungi have been addressed simultaneously. The consensus of multiple studies in which endophytic and epiphytic fungi were both sampled, is that surface and interior communities are very different from each other, with many species strongly favoring surface or interior

colonization, often exclusively one or the other (Zambell and White 2014, Cabral 1985, Santamaria and Bayman 2005). Studies of greenbrier showed that the Bray-Curtis metric of community overlap between surface and interior was 20% for stems in winter, 15% for stems in summer, and 22% for leaves in summer (Zambell and White 2014, Chapter IV). Thus, while mostly separate, typcially some species overlap slightly into the other sphere. There also appear to be a few species that have a strong presence in both. I have found that an *Anthostomella* species was common on both surfaces and interiors of both stems and leaves of greenbrier in summer (Chapter IV). Micro-habitat usage may change temporally as well. I found that *Aureobasidium pullulans* was a frequent colonist of surface and interior tissue of greenbrier stems in late winter (Ch. III). In summer, it became much more heavily skewed towards surface colonization, but still had an endophytic presence (see Ch. IV). In a study of eucalyptus leaves, Cabral (1985) observed a *Coniothyrium* sp. that began as an epiphyllous species, but then migrated to internal tissue in a different season. A switch from surface to interior has been observed in few other cases close to senescence (Osono 2006).

Some studies have examined how ecological variants might affect both endophytes and epiphytes, including the effect of position of leaves within a canopy (Osono and Mori 2004), and the effect of seasonality and age of leaves (Cabral 1985, Osono 2008), the effects of spatial proximity and of position along the axis of the stem (Zambell and White 2014), and the effect of tissue-type and geography (Chapter IV). Comparison of both communities in the same study has supported the proposition that endophytes are more influenced by the physiological variables internal to the plant (Cabral 1985, Zambell and White 2014). For example, Cabral (1985) noted that

endophytic species showed more dependence on age and physiological condition of leaves than epiphyllous species. Regarding the question of interaction between surface and interior fungi, Zambell and White (2014) (i.e. Ch. III) did not find any evidence of correlations between surface restricted fungi and interior restricted fungi. Yet some species that spanned the surface and interior did show positive correlations with more restricted species, which may indicate an interaction.

For some purposes, it may be justifiable to omit the study of epiphytic fungi from endophytic investigations. However, in future cases involving investigations of (1) modes of dispersion, (2) mutualistic effects, or (3) competition of any sort involving secondary metabolites, investigators should not draw conclusions about purely endophytic interactions without knowledge of surface fungi in the system under review.

Endophytology

Petrini (1991) dates the beginning of broad interest in endophytology to Bernstein and Carroll's (1977) study of Douglas fir (*Pseudotsuga menziessi)* needles. In the study, it was shown that healthy needles of many trees within a watershed were consistently infected with three recurring fungal morphotypes in all needles over three years old. Broader endophytic surveys of European conifers (Carroll et al. 1977), conifers of the Pacific Northwest (Carroll and Carroll 1978), and broadleaf evergreen shrubs of Oregon (Petrini et al. 1982), showed that endophytes were not only ubiquitous in woody plants, but that different plants had characteristic taxa, at least to the family level or lower. More recent research indicates that different species of forbs too may have characteristic, if not host specific, fungal taxa associated with them (Wearn et al. 2012).

The term 'endophyte,' according to Hyde and Soytong (2008), is most commonly used according to Petrini's (1991) definition as "…all organisms that at some time in their life, can colonize internal plant tissues without causing apparent harm to their host." In practical terms of isolation, Cabral et al. (1993) call endophytes "…any fungi isolated from internal symptomless plant tissues." Other definitions that substantially vary from this add some aspect that is difficult to prove without further study beyond isolation, such as that endophytes are mutualists, or that they do not cause disease, or that they do not occupy surfaces (Hyde and Soytong 2008). These seem to be unjustified assumptions as the experience of researchers indicates that endophytes are likely part of a continuum of life-styles (Schulz and Boyle 2005); habitat and ecological function may change based on environmental conditions, developmental stage, and the individual genes of host and endophyte. Finally, it is important to distinguish that mycorrhizae, although they occupy internal tissues of roots, are considered a separate ecological class of organisms from endophytes as they are commonly understood (Arnold 2007). Some endophytic fungi are distributed in both aerial tissues and roots. However, it has been shown that there is a strong distinction between root vs. shoot and leaf communities (Wearn et al. 2012).

Rodriguez et al. (2009) attempted to refine classification of endophytes into four different categories. **Class I** endophytes, in this scheme, are clavicipitaceous endophytes of grasses, a group of phylogenetically related, vertically transmitted, systemic, host specific fungi that have been shown to alleviate both abiotic and biotic (herbivory) stresses of their grass hosts, and thus are considered to be mutualistic (Kuldau and Bacon 2008). **Class II** endophytes were described as a group that can be phylogenetically diverse, but that are very dominant within a plant, showing extensive colonization of all

tissues including roots, shoots, and leaves, being vertically transmitted perhaps via seed coat, and conferring often habitat-specific, mutualistic benefits. **Class III** endophytes, the main focus of this review, are the default aerial group of fungi found in all plants. These are characterized as phylogenetically diverse, of rich *in planta* diversity, of typically locally very limited infections, and horizontally transmitted for the most part. Systemic growth, presumably necessary for vertical transmission, seems to be uncommon or absent in most genera of non-clavicipitaceous endophytes, (Boyle et al. 2001, Stone et al. 2004, Yan et al. 2015). However, Yan et al. (2015) suggest that systemic growth may commonly take place but only at the plant seedling stage. Rodriguez et al.'s (2009) Class II endophytes can belong to some of the same genera (e.g. *Fusarium*, *Colletotrichum*, *Cryptosporiopsis*) as Class III endophytes, and so are difficult to distinguish from Class III without further study demonstrating systemic colonization or habitat adaptation. In other words, Class III can be taken to be the most common endophytic association and the expectation barring further information, while Class II are the special case involving an extensive systemic growth and high mutualistic potential. Finally, **Class IV** endophytes are dark septate root endophytes—a group confined to roots, though non-mycorrhizal, and not discussed further here as this chapter focuses on aerial endophytes.

The study of endophytes has generated a very large body of literature (>1000 papers about non-clavicipitaceous endophytes between 1970-2009; Rodriguez et al. 2009), combining ecological questions with basic surveys of various plants, which can be daunting to both newcomers and even those experienced in the field. The subject has been reviewed and/or analyzed from a variety of perspectives since the late 1980's, including publications by Carroll (1988), Petrini (1991, 1996), Stone et al. (2004), Shulz

and Boyle (2005), Schulz and Boyle (2006), Arnold (2007), Sieber (2007), Saikkonen (2007), Hyde and Soytong (2008), Rodriguez et al. (2009), Porras-Alfaro and Bayman (2011), Unterseher (2011), and Suryanarayanan (2013), Weber and Anke (2006) (not including reviews that only focus on clavicipitaceous endophytes). Despite the many reviews, endophytes often take on, in the words of Hyde and Soytong (2008) an almost mystical aspect. Instead of emphasizing the mysterious and unpredictable aspects of endophytes, the first goal in the present review is to synthesize *what is known* of how most endophytes gain nutrition and reproduce, with some examples, as basic background for how community interactions and community assembly might take place. After establishing some basic natural history, we can move on to how to better understand community ecology in this group. In the following sections we discuss endophytes' relationship to the basic roles expected of fungi as heterotrophic plant associated organisms: pathogens/parasites, saprotrophs, commensal scavengers, and mutualists.

2. Review: basic nutritional and reproductive strategies of endophytes

Are endophytes pathogens?

Some proportion of endophytic infections can undoubtedly be considered latent pathogens. Sinclair and Cerkauskas (1996) in discussing this topic, listed sixteen fungal pathogens that can have a latent infection period in a variety of crops, including fungi of genera *Alternaria*, *Botryosphaeria*, *Botrytis*, *Cercospora*, *Colletotrichum*, *Cryptosporiopsis*, *Dothiorella*, *Guignardia*, *Lasiodiplodia*, *Leptosphaeria*, *Monilinia*, *Phomopsis*, and *Pyricularia*. Some of these have long dormancy periods, so that absent the pathologist's knowledge, an uninformed endophytic surveyor would not know they have any pathogenic effects. For example, the pathogen *Leptosphaeria maculans* (Desm.)

Ces. & de Not.) infects oilseed rape (*Brassica napus* L.) through leaves in autumn, grows into stems systemically, and then forms cankers in early summer (Hammond et al. 1985). Lag time between infections and lesions was shown experimentally to range from 77 days to 175 days depending on temperature (Hammond et al 1985). Another example of dormancy can be found in *Pyricularia grisea* (Cooke) Sacc., the cause of pitting disease of bananas. The fungus forms an appresorium to penetrate the cuticle of young banana fruits, and may remain asymptomatic in the cuticle for months, beginning destructive activity only at or after harvest time (Meredith 1963).

So how large a proportion of the endophytic community can be explained by these types of organisms? Cases in which both latent pathogens and endophytes were both studied suggest that they do not make up the majority. First, consider the case of *Phomopsis viticola*. *Phomopsis* is a genus that is very commonly encountered both in endophytic surveys, and as a genus of problematic pathogens (Sieber 2007, Udayanga et al. 2011). Pscheidt and Pearson (1989) demonstrated that the pathogen *Phomopsis viticola* inoculated into clusters of Concord grape vine at the time of bloom or shortly after caused an increase in later losses compared to controls, demonstrating a long dormancy period from bloom to harvest. They also demonstrated that *P. viticola* caused latent infections using the method of treating inoculated fruits with paraquat. Subsequently, several studies have assessed the infection frequency of this species among other endophytic infections. Mostert et al. (2000) found that it was present endophytically in a vineyard, and was pathogenic as verified by Koch's postulates. However, the pathogen was not a dominant endophyte in grape plants, but might be considered a low frequency but consistent, species, accounting for only 3% of total endophyte isolates (or

9% of the frequency of the most common species). Gonzalez and Tello (2011) in an multi-site, multi-year, multi-cultivar study of grape vines in central Spain, also found *P. viticola* was a consistent presence across sites, but not particularly common, making up only 1.53% of all endophytic infections recorded. Gonzalez and Tello (2011) remarked that *P. viticola* was the most common of all grapevine pathogens recovered in that study, which also recovered in lower frequencies causal agents of Petri disease (*Phaeomoniella chalmydospora*, *Phaeoacremonium aleophilum*, *Phaeoacremonium inflatipes*), and Black Foot (*Cylindrocarpon destructans*).

The results of Photita et al.'s (2001, 2004) studies of banana leaves may also help to put the clearly pathognic component of endophytes into perspective. An endophytic survey of asymptomatic leaves of banana plants yielded many fungal species, including *Deightonella torulosa* (Photita et al. 2001). When inoculated onto excised banana leaves in wet chambers, the *D. torulosa* isolates caused the formation of leaf spots indicating pathogenic potential (Photita et al. 2004). The spots formed whether inoculation was via spore suspension or agar plug, and with or without wounding, but did not occur in controls or with other isolates (Photita et al. 2004). Despite the pathogenicity of *D. torulosa*, it is worth noting that no other dominant endophytes from this survey were found to cause disease symptoms suggesting that many endophytes are not pathogenic. It is also notable that the apparent pathogen, *.D. torulosa,* was not ubiquitous as an endophyte in *Musa* leaves. Instead, it was very dominant at one site, rare at a second site, and undetected at three other sites in the same national park in Thailand (Photita et al. 2001). Again, this suggests pathogens are a small part of the endophytic community.

In a third example, Ganley et al. (2004) explored the connection between pathogens and endophytes in a tree that is well-studied from a pathological perspective, *Pinus monticola*. They found that the majority of endophytes (90% of isolates) of *P. monticola* were of family Rhystimataceae, a family in which there are also three known parasites of the tree. Yet, none of the endophytes were most closely related phylogenetically to the three known rhystimataceous parasites of the tree. Instead, many were most closely related to, yet distinct from, parasites of other *Pinus* species. Some fell into clades with no known close relative, and one clade was most closely related to a saprobe of various *Pinus* species, including *P. monticola*. A similar pattern was also found for *Mycosphaerella* and *Rhizosphaera* endophytes of *P. monticola,* which were most closely related to, but morphologically distinct from, pathogens of other *Pinus spp.* (but not to pathogens of *P. monticola*).

From these examples, we can conclude that highly damaging fungal pathogens are infrequent in endophytic assemblages. However, many endophytes may be in a twilight area between pathogens and saprotrophs, mainly attacking tissue that is at the end of its life. In a good example of this, Weber and Anke (2006) reported that *Phoma medicaginis* was the dominant endophyte of *Medicago* spp., but that only very limited symptoms of infection appeared both in the field and in artificially inoculated plants. Dried leaves from either source, however, upon incubation became covered with sporulating *P. medicaginis* pycnidia demonstrating that this very weak pathogen has potential to reproduce saprotrophically without ever having manifested pathogenic symptoms. Endophytes that cannot be re-isolated from litter are often speculated to be weak parasites. For example, Koide et al. (2005) noted that *Colletotrichum gloeosporioides* was isolated

endophytically from living leaves, but not from litter in both *Camellia japonica* and *Swida controversa* (Osono et al. 2004) and suggested the species is parasitic. A *Coniothyrium* species that Cabral (1985) observed migrating from *Eucalyptus* surface to interiors over the course of the season, was observed to only sporulate on attached mature, senescent, or dry attached leaves, but was not observed in fallen litter. This may be considered weakly parasitic, but hardly pathogenic. Finally, quiescent pathogens that resume growth only as fruit ripens (Prusky 1996) also border between pathogen and saprotroph, depending on whether they actually have any impact on plant reproduction by damaging seeds or deterring seed dispersers.

Some distinction should also probably be made between latent disease that will commence pathogenic development during the proper season or phase of the plant's life-cycle, versus opportunistic infections in which disease only develops under occasional conditions. Slippers and Wingfield (2007) have argued that *Botrysophaeria* diseases, which can be very destructive, are only caused in situations of suboptimal growth conditions, or some other stress; and that in nature *Botryosphaeria* endophytes may play a beneficial role in abiotically stressed, or insect-infested, tissues, which again borders on saprotrophic. Finally, Sieber (2007) has pointed out that *Fomes fomentarius* and *Nectria coccinea* are pathogenic species that have been proven to be frequently present endophytically within healthy trees, while disease is only triggered by certain stresses. If the tree does not encounter the needed stress to trigger disease then these endophytic infections might become extinguished without reproduction.

Are endophytes decomposers?

Overwhelming evidence indicates that some endophytes play a role in decomposition of tissues they inhabit. Endophytes isolated in agar are by definition able to survive independently of a living host. Furthermore, surface disinfected segments of leaf and stem tissue placed in wet chambers produce sporulating fungi that correspond to the endophytes isolated in agar. However, intact whole leaves or large stem cuttings, able to remain alive in wet chambers, do not produce endophytic growth (personal observations). He et al. (2012) showed that all 39 species of endophytes isolated from senescent *Cinnamomum camphora* leaves showed leaf decomposing ability in pure culture. Sun et al. (2011) showed that common endophytic isolates tended to have higher decomposing ability than rare isolates on the same host, suggesting not only ability to decompose, but adaptation to decomposition of the host tissue. Of 21 endophytes tested, many produced enzymes that could be used in decomposition—protease and lipase were most common (from 19 spp.), followed by laccase, amylase, cellulase, pectinase, pectate transeliminase, and finally, tyrosinase the least common (10 spp.).

Many studies historically have examined succession of phyllosphere species from living to dying leaves or litter, using surface sterilization, and surface washing to segregate endophytes from epiphytes (Macauley and Thrower 1966, Kendrick and Burges 1968, Ruscoe 1971, Watson et al. 1974, Wildman and Parkinson 1978, Cabral 1985, Osono 2002, Osono et al. 2004). However, phyllosphere species are difficult to distinguish based on spore morphology alone, and it might be argued that similar morphotypes in litter and leaves do not prove that these are the same species. Promputtha et al. (2007) dispelled doubts of this nature by showing phylogenetic evidence that

several endophytic live leaf isolates and saprophytic litter isolates of *Magnolia lilifera* leaves were the same, including species of *Colletotrichum*, *Phomopsis*, *Fusarium*, and *Guignardia*. Further, Promoputtha et al. (2010) went on to show that litter and endophytic isolates were able to produce identical enzymes.

Osono (2006) reviewed the literature on the role of phyllosphere species in decomposition, and estimated that about 2/3 of phyllosphere species (64% of epiphytes, 67% of endophytes) are also present in leaf litter, but with much variability in different plants). In some cases, species that are known to reproduce in litter are not re-isolated in litter for unknown reasons. For example, Unterseher et al. (2013) isolated *Discula umbrinella* from European beech leaves but not litter. Yet they noted that it is well documented that teleomorphs of this species appear in spring in the previous season's beech litter. Since endophytes and epiphytes show evidence of niche partitioning by season, it may be that certain species are unable to survive or compete in litter during certain seasons and become extinct or undetectable in litter at those times. The literature indicates a trend of decreasing proportion of phyllosphere species as decomposition progresses, with many species typically exhausting their life cycle within the first year of leaf senescence (Osono 2006). The absence of some endophytes from litter, and the decrease at later stages, may be taken to suggest that endophytes are generally not strong competitors in the saprophytic environment. Some endophytes also may be able to compete if previously established, but cannot invade litter if they were not there in the endophytic stage (Koide et al. 2005, Osono 2002).

One well-studied example of an endophytic life strategy, perhaps representative of many horizontally transmitted aerial endophytes, is that of *Rhabdocline parkeri.* The species is ubiquitously associated with Douglass-fir needles in Western Oregon, and the number of infections increases with needle age, ranging from 0.2 to 20 infections per mm^2 (Sherwood-Pike et al. 1986, Stone 1987, Stone et al. 2004). The infections are confined to a single host cell, which dies upon infection, leaving a small thallus that lies quiescent until senescence of the needle, when the fungus resumes expansive colonization (Stone 1987, Sieber 2007). The endophyte only sporulates in needles that are dead, senescent, or galled by insects (Sherwood-Pike et al. 1986). There are multiple examples of endophytes having a similar life-cycle, in which growth and production of fertile reproductive structures ensues upon senescence and death, including *Lophodermium piceae* in *Picea abies* needles*, Coccomyces nipponicum* on *Camelia japonica* leaves, and *Mycosphaerella buna* in *Fagus crenata* leaves (Osono 2006).

Non-destructive life-cycles in living tissues

Could some common aerial endophytes mirror aspects of the clavicipitaceous endophytes, reproducing and spreading without damaging host tissue or requiring death of host tissues? It has been shown that a sterile, non-clavicipitaceous endophyte of basil, can form epiphyllous nets (Mucciarelli et al. 2002). This species could conceivably draw nutrition from the plant interior, while dispersing propagules via epiphyllous nets, a mode of reproduction also recently demonstrated in the Clavicipitaceae (Tadych et al. 2012). However, this should probably be considered an exceptional species, one of Rodriguez et al. (2009) Class II endophytes, as it is systemic and strongly enhances the plant's growth.

In an example of more common, non-specialized fungi, Hodgson et al. (2014) showed that endophytic strains of *Alternaria alternata* and *Cladosporium sphaerospermum* can be recovered from sterilized seeds of multiple forb species, and are able to transmit to new cotyledons and first leaves in axenic conditions. These fungi were shown to infect pollen endophytically, and it was suggested that they may enter the ovule via pollen tube to infect the interior of seeds. It is also possible that these species have an epiphytic presence, and simply transfer via growth onto and into seed coats (Hodgson et al. 2014). The role of epiphytic growth is generally neglected in studies of endophytes, but may play a major role in the life cycle of those endophytes that also have an epiphytic presence, such as *Aureobasidium pullulans* (see Ch. III, Ch VI). As a common mold it is likely that *A. pullulans* can subsist on sparse nutrients and produce conidia on the surface of a plant without damaging the plant. In conclusion, those endophytes that show an expansive epiphytic presence, might not require digestion of weakened or dead host tissues for reproduction—on the other hand, as explained in the sections above, the majority probably do at some time in their life-cycle digest plant tissue.

Endophytic dead ends?

Microbial propagules are formed in vast numbers, and different habitats in the form of various plant species sit side by side receiving the same influx of spores. The fact that an endophyte has invaded a plant should not lead to the assumption that there is some tightly co-evolved relationship between the particular plant and fungus, especially if the endophytic isolate is uncommon in the plant. Many generalist fungi are commonly isolated as endophytes (e.g. *Aureobasidium pullulans, Cladosporium cladosporioides, Cladosporium sphaerospermum, Alternaria alternata;* Zambell and White 2014,

Hodgson et al. 2014) proving that the ability to colonize internal plant tissues can be a general, non-host specific trait. Fungi with traits allowing endophytic infection may still lack other adaptations that would allow them to survive, or complete the life cycle in a given host.

Many host-endophyte combinations could be dead end paths for the endophyte, though there is currently limited evidence to prove this principle in the form of specific life histories. One example was given by Petrini (1996), who described the case of *Hypoxylon fragiforme*, a known saprotroph that sporulates on wood of beech trees, and is a characteristic endophyte of beech trees. The fungus is also isolated endophytically from trees that it is not known to ever sporulate on; for example, *H. fragiforme* was recently recorded in *Pinus sylvestris* needles (Persoh 2010), despite that sporulating forms are unknown in conifers (Unterseher et al. 2013). Petrini (1996) used the term "expression specificity," to indicate that the fungus only forms ascospores on a subset of hosts, suspending judgment as to what it is doing in other hosts. It is possible that these infections find some hidden way to reproduce, producing inconspicuous undiscovered propagules, but also possible that they are dead ends for the fungus. It has been demonstrated that *H. fragiforme* ascospores remain dormant until detecting chemical cues derived from host tissue that induce germ tube formation (Petrini 1996). The fact that this species appears to infect so many non-hosts, despite having a mechanism of host recognition, may be demonstrative of the very imperfect nature of host-endophyte pairings in nature. Furthermore, most endophytic propagules germinate on agar or nutrient solution without any host specific cues (personal observations). Shulz and Boyle (2005) also discussed the idea that some endophytes cannot survive long-term, calling

them "incidental opportunists," and referencing dung associated fungi found as endophytes. Whether discussing "incidental opportunists," "expression specificity," or "endophytic dead ends," I emphasize that there may be many random encounters in the endophytic world that do not involve well tuned host-symbiote relationships.

Mutualism

The fact that many endophytes play ecological roles involving saprotrophy does not mean that they do not provide positive effects for plants under some circumstances. Competitive self-interest of endophytes in some cases may also be beneficial to the plant, in competitive exclusion of virulent pathogens from dead or weakened material, or decreasing insect infestations via destruction of infested tissue (Carroll 1988). A direct insect anti-herbivory effect in living tissue has been linked to taxonomically diverse foliar endophytes of *Allium* herbs (Muvea et al. 2014), and cotton (McGee et al. 2002). The subject of non-clavicipitaceous mutualisms in regard to plant stress and growth has been reviewed by Yuan et al. (2010).

Saikonnen (2008) has pointed out, rightfully, that rather than classify endophytes as mutualist organisms, it is better to see them as competing plant consumers. In some ecological circumstances their effects may be positive to the plant, at other times negative. As recently summarized by Ridout and Newcombe (2015), it has been shown in many studies that a variety of phyllosphere species, both endophytes and epiphytes, may either reduce or enhance the effects of plant disease, e.g. disease reduction (Arnold et al. 2003, Dingle and Mcgee 2003, Perello et al. 2002, Clarke et al. 2006, Ganley et al. 2008, Istifadah and McGee 2006, Raghavendra and Newcombe 2013, Lee et al. 2009, Andrews

et al. 1983), disease increase (Morin et al. 1993a,b, Busby et al. 2013) and different effects by different species (Kurose et al. 2012, Ridout and Newcombe 2015). Once community structure and assembly is better understood, it may be possible to use this knowledge to increase the abundance of beneficial or detrimental phyllosphere species according to the need. It also makes sense in some applications to study and measure mutualistic potential of different endophytes. However, when asking basic questions of community assembly, it is best to view endophytes mainly as consumers and put aside the question of mutualism for the time being.

3. A framework for future study

Environmental niche adaptation and competition

Natural communities are thought to be organized by a combination of niche and neutral forces (Gravel et al. 2006). Niche differentiation allows for species to coexist because they are specialized to either slightly different environmental conditions, or have different lifestyles. For this reason they should compete more strongly with each other than with other species. In neutral organization, forces of extinction and migration may allow for coexistence even though species traits are about the same. In endophytic research, several experimental studies have clearly demonstrated that plants with different genetic traits planted together, select for different endophytic communities (Balint et al. 2013, Saunders and Kohn 2009). Such environmental-based filtering argues against neutral assembly towards greater importance of niche based organization (Saunders and Kohn 2010). For this reason, studies which lump all species as equal, only measuring species richness, or total colonization, are probably missing important information about individual species differences.

One of the main successes in studies of endophytes has been identifying environmental variables that seem to be important for selecting particular endophytic fungi. These can be considered environmental filters, or important niche axes. While many studies have shown that environmental conditions may somehow significantly affect fungal communities, it is more interesting when certain conditions are shown to favor one or more dominant endophytes, while disfavoring others. Otherwise, it might just mean that certain conditions are favorable for fungi in general. A major determinant of endophytic communities is plant host species, which has been shown, in mixed co-existing groups of plants, to clearly select for different dominant endophytes (Suryanarayanan et al. 2000, Sun et al. 2012, Unterseher et al. 2007, Persoh 2013). Other niche axes, that is, factors that clearly favor different dominant endophytes, are tissue or organ type (Wang and Guo 2007, Kumar and Hyde 2004, Sun et al. 2012, Chapter IV), season (Tadych et al. 2012, Mishra et al. 2012, Unterseher et al. 2007), tissue age (Hata et al. 1998, Osono 2008), sun vs. shade (Unterseher 2007), and presence or absence of a defensive compound (Saunders and Kohn 2009). Finally, in a few cases it has been shown that samples taken at regular intervals from the base to the tip of a plant organ show gradients of preference for certain endophytes (Zambell and White 2014; Hata and Futai 1995). Upon viewing the shifting zones of dominance in endophytic habitats, the question naturally arises as to what mechanism creates this.

The Hutchinsonian concept of fundamental vs. realized niche is probably a good starting point for talking about these different zones of dominance by different endophytes (Hutchinson 1978). In this concept, each species has particular environmental requirements that can be represented as axes in an abstract hypervolume. These are the

conditions necessary for survival of the organism, and may include suitable food sources, shelter conditions, temperature, pH, etc. The plant interior could be a part of the fundamental niche of a great many endophytic species. However, despite adaptation to certain conditions, a species may be excluded from certain spaces by a better competitor, so that its realized niche is smaller than its fundamental niche. The question then is, are the observed frequencies of endophytes the result of fundamental niche filling, or are they the realized niche after competitive interactions? This concept was famously demonstrated by Connell's (1961), study of two barnacle species that inhabited different habitats in the rocky intertidal zone. One species was able to occupy all zones if the competitor was removed, but in the face of competition was constrained to inhabit only the higher, drier areas of the shoreline, to which its competitor was poorly adapted.

While it is tempting to look for an analogy of endophytic communities in other sedentary communities like that of Connell's barnacles (1961), due to life-cycle differences in endophytes, when and how competition takes place between them might be very different. Competition may or may not take place in living plant tissues (discussed further in part 4). To examine how the interplay of environmental niche and competition may shape endophytic communities, it will be necessary to break down the endophytic life-cycle into component parts so that we can quantify dynamics of endophytic community assembly and set up biologically meaningful experiments.

Quantifiable aspects of the endophytic life-cycle

Based on the literature reviewed above, it is reasonable to assume that most common aerial endophytes colonize plant tissues at some opportune time, lie in a state of

constrained, localized growth within internal tissues, then resume growth at a time of either (a) high plant stress or (b) during seasonal or age-related tissue senescence. Most endophytes must reproduce in tissue that is stressed, dying, or dead.

As a consequence, it is important that mycologists keep in mind the two main periods of life for typical, Class III endophytes, being (A) the colonization/latency phase, and (B) the often ignored, growth/reproductive phase. A more detailed breakdown of this cycle, with quantifiable processes, can be represented as five successive phases with measurable qualities: (1) The rate of appearance of dispersed propagules on living plant tissues → (2) the rate of successful internal colonization per propagule added → (3) survival/mortality rate of infections in living tissue (fraction of originally successful colonies that die per unit time) → (4) competitive ability of a fungal species in dead or weakened tissue → (5) rate in generating new propagules → (1) repeat cycle

By experimentally dissecting and quantifying these aspects of the endophytic life-cycle for different species, it should be possible to predict whether some species should become super dominant within a particular plant or tissue, others rare, and where environmental tolerance vs. competitive interactions shape the community. First consider the latency period. Phases (1) dispersal, and (2) internal colonization, could be glossed over for the time being in favor of a single measurement in the field of successful new endophytic colonies established per unit time. Combining this measurement with experimental measures of phase (3), the mortality rate of infections, should allow one to predict the concentration of infections *in planta* for each endophytic species.

The ability to measure these steps, and the fact that they are important, has been demonstrated. Shulz and Boyle (2005) described how host-derived endophytic strains were more frequently re-isolated compared to non-host strains at 21 days after inoculation. This demonstrates that the non-host endophytes had either a lower rate of establishment or else a higher rate of mortality once established, or both. Suryanarayanan (2013) described an unpublished study in which a *Trichoderma* sp. derived from marine algae was inoculated into multiple crop species. Recovery rate dropped from around 90% at 7 days post-inoculation to 20-30% after 28 days. This shows that mortality of endophytic infections takes place over the course of several weeks. For infection rate, Arnold and Herre (2003) placed endophyte free plants in the field and measured their density of endophytic colonization at 7 and 15 days, though this measure ignores mortality rate. The rate of new infections could be disentangled from colony mortality by frequent replacement of fresh plants after rain events or at regular, short time intervals.

4. Experiments in competition

Competition in dead or dying tissue

Since many endophytes resume growth at plant senescence, competition may become intense during this phase. In wet chamber experiments with autoclaved plant tissues it can be observed that many endophytes will expand to colonize all available plant tissue if allowed (personal observations). In densely colonized tissues, competition with other endophytes and surface colonists must be unavoidable for space and nutrients. We already know that certain species show higher frequency in particular tissues, or particular plant species. What is unknown is whether high frequency also translates to competitive dominance once the tissue is dead. There are several different ways this

might play out. In one scenario, (1) dominant species in colonization may also be competitively superior in tissue they tend to dominate, overgrowing and eliminating ill adapted rare morphotypes as the tissue dies, and reducing species diversity; or alternately, (2) common endophytes might have no competitive advantage over less common endophytes in dead tissue, and may even be inferior as competitors in dead tissue. If this is the case the biomass of rare species may become proportionally greater in dying tissue; or (3) there may be subtle variations, where dominant endophytes are superior under the right season, or the right conditions of senescence, but become eliminated if tissue dies under different conditions.

Supporting the idea that tissue colonization dominance does not translate to competitive growth dominance, it has been shown that some litter endophytes are unable to compete against forest floor saprotrophs unless they have a previous endophytic foothold (discussed previously). Similarly, some endophytes may rely on high colonization and quick growth and defense of an area, or quick reproduction, before other aggressive endophytic strains can overcome them. Also, supporting scenario (2) or (3), Douanla-Meli et al. (2013) found that *Mycosphaerella* endophytes were common in healthy leaves of *Citrus limon*, but this did not translate to competitive dominance in weakened tissue, as they became rare in yellowing leaves. *Colletotrichum gloeosporioides*, on the other hand became more common in yellowing leaves than in healthy. From these observations alone, it cannot be known whether the environmental changes between green and yellowing leaves killed off *Mycosphaerella* infections, or if they were eliminated by competitive interactions that ensued upon leaf yellowing. Was this *Mycosphaerella* sp. better suited to a different tissue, a different mode of senescence,

a different season of senescence? This study also raises questions about when competition ensues between endophytes? It may occur in living tissue, slightly senescing, yellowing or weakened tissue, or only at an advanced stage of senescence and death. Basic questions about competition involve the outcome of competition between rare and common species, host-specific vs. generalist endophytes, and weak pathogens vs. commensal saprotrophs, and under conditions of seasonal senescence vs. stress induced weakening of the plant.

It is clear that we really have no idea of how competition plays out between endophytes as tissue senesces. To answer these questions, researchers will have to design experimental systems in which they can pit different endophytes against each other under different conditions and combinations and observe the dynamics from living to dead tissue. It would be ideal if experimenters could design microcosms using herbaceous plants that have a fast life cycle. The entire process, then from inoculation in living tissue to senescence and decay could be studied. With enough skill, microcosms could be designed in which multiple successive endophytic life cycles might be completed by leaving litter in the system, and allowing reproduction from seed. More simply, detaching leaves of endophyte inoculated plants and incubating in vitro might serve for some experimental objectives.

Experiments involving single vs. multi-species plant assemblages might also help explain whether endophytic diversity is dependent on plant species diversity. The role of dispersal efficiency can also be examined by varying the exposure of microcosms to different degrees of rain splash.

Competition in living plant tissues

While it is generally accepted that at least some endophytes compete in a saprotrophic phase, a major unanswered question in endophytology is whether species compete in living plants. Using experimental microcosms it should be easy to test whether the presence or absence of one endophytic species *in planta* affects colonization and recovery rate (via sampling) of a second species; though experiments would have to be well designed to prevent artifacts of overgrowth in sampling methodology. My own research showed that certain species peaked in different zones of height along the length of greenbrier stems (Zambell and White 2014). Direct chemical interference between them could be an explanation for the pattern, similar to Connell's (1961) barnacles.

While it is easily imagined that competition is absent in the latent phase, there is some suggestive evidence that it may actually be important. Saunders and Kohn (2009), found that *Fusarium* isolates were up to 35x more frequent in leaves of maize cultivars that produced benzoxazinoid (BX) toxins compared to a natural mutant that did not, planted in the same field; they also showed that *Fusarium* isolates are resistant to this toxin in vitro. The authors suggested that competitive dynamics among endophytes were altered, giving *Fusarium* an advantage. It is hard to imagine how else the addition of a toxic compound could increase the presence of a fungus. The only other explanations are that *Fusarium* might use the toxin as an environmental cue for attachment or germination, or that there is some unknown physiological correlate to the loss of the toxin. If competitive dynamics were at play, then the exact nature of this should be determined. The possibility of an epiphytic presence should not be ignored. *Fusarium* species appear to make up a significant proportion of the phylloplane mycoflora in maize and other

plants (Caretta et al. 1985, Ahmed 1986, Asensio et al. 2007), making epiphytic competition more important than it would be for a more endophytically restricted species. A BX mediated change in competitive dynamics may have been restricted to the epiphytic plane, as the toxin also would have probably impacted the bacteria, yeast and filamentous epiphytic populations on leaf surfaces (BX toxicity is not restricted to fungi; Adhikari et al. 2015). Decreased competition for nutrients such as pollen (Last and Warren 1972), or reduction of direct interference competition with phylloplane species may have allowed for a higher number of *Fusarium* propagules to successfully invade and spread across surfaces and consequently establish endophytic infections.

In another relevant experiment, Mohandoss and Suryanarayanan (2009) added an exogenous agent of chemical selection, a fungicide hexaconazole, and observed changes to the endophytic community following the spray period. Some species, such as a xylariaceous morphotype, seemed to grow in frequency in the post spray plants more so than the control plants——the authors suggested that competitive release may have played a role in community changes. Obviously there is a need for more research into the possibility of competition in living plants, but as stated above, the surface must also be studied to understand whether it is a purely endophytic interaction or whether surface hyphal interactions could be involved.

The production of antagonistic chemicals by endophytes is not the only way that localized internal infections might compete. A mode of indirect competition that is possible among endophytes could be by triggering a change in plant chemistry that is more detrimental to competitors than it is to the species that induces the change

(Saunders and Kohn 2010). Several studies have shown that endophytes can influence of plant chemistry. Mucciarelli et al. (2007) showed that an endophyte of *Mentha piperita* (Lamiaceae) influenced both total quantity and proportion of main components in essential oils. This example seems to be more representative of special, Class II type endophytes—it is a sterile mycelium identified to class Pyrenomycetes, a systemic fungus that forms hyaline epiphyllous nets on meristems of peppermint, extends to roots when grown in vitro, and has been shown to strongly stimulate plant growth (Mucciarelli 2002, 2003).

A more generalizable effect of mixed endophytes, however, on essential oils has also been demonstrated in the plant *Atractyloides lancea,* a medicinal asteraceous plant. It was shown that two different endophytes (*Gilmaniella* sp., and *Cunninghamella* sp.) had different effects on essential oil composition (Yang and Dai 2013). That study demonstrated competition and priority (order of addition) effects between two endophytes. However, the methodology of the study used plant tissue culture inoculated with plugs of fungal mycelium on PDA, making the translation of the results to field-grown plants questionable. Tissue culture produces a high humidity environment and may promote excessive fungal growth compared to open-air conditions. Furthermore, competitive effects may have taken place outside of the plant interior, as inoculation by PDA plug implies some pre-colonization expansion of hyphae and interaction between the two species. The effects of endophytes on essential oils should be tested using more natural conditions in soil and open air, and inoculation via spore suspension.

Finally, Estrada et al. (2013) found evidence that ants could detect endophyte-mediated differences in chemistry that eluded human instrumental analysis. They inoculated *Cucumis sativa* seedlings with high and low densities of the endophyte *Colletotrichum tropicale*, and tested the influence of endophyte density on the ants' food preferences. The ants cut about a third more leaf area from plants that had been inoculated at a low vs. a high density of endophytes. When paper discs were impregnated with plant extracts from the low and high endophyte density plants, the ants carried off more of the discs made from the low endophyte density plants. Some element of the leaf extract must have influenced the ants' preference, but researchers were unable to detect differences in either volatile compounds, cuticular waxes, nutrient content, water content, or specific leaf area between high and low density colonized leaves. It was speculated that some undetected, low volatility compound, was responsible.

5. Community patterns

Community structure and variation in endophytic communities

Endophytic communities tend to be species rich, and the process of sampling in endophytic surveys is rarely completed to the point where all rare species have been inventoried (Unterseher 2011). Thus, a long tail of rare species is expected in endophytic species sampling, though it need not always be catalogued depending on the objectives of study, which often may involve characterizing ecological aspects of the dominant species. The difficulties of exhaustive sampling, species richness estimators, and comparison of species richness between studies has been discussed by other authors (e.g. Unterseher 2011).

One consistent aspect of endophytic community structure is the strong dominance of one or a few species, which was apparent from the early studies of endophytes in the 1970s, which often showed a drastic drop in abundance between the most dominant species and those that were intermediate to rare. For example, Petrini et al. (1982) found *Arctostaphylos uva-ursi* was dominated *by Phyllosticta pyrolae*, which made up 16.3% of isolates, while the next five most common species fell between 1 and 4% of isolates. *In Mahonia nervosa* the top three species were *Leptothyrium berberidis* (53.4% of isolates), *Septogloeum* sp. (13%), then *Phomopsis* sp. (2.4%). In some cases, several species were more codominant, as for example *Abies amabilis* needle petioles (the very base of the needle) had similar proportions of three species at 28%, 25%, and 19%. Sieber (2007) in a review of forest tree endophytes, was able to list typically 1-2 (maximum 5) dominant species of endophyte for each of 52 species of tree that have been surveyed. The ease with which dominant species can be picked out from endophytic communities seems to be a strong recurring characteristic. There has been no formal comparison of endophytic and epiphytic fungal communities to those of other host-colonist systems to our knowledge. Looking, for example, at frequencies of vascular plant epiphytes on various tree species in the neo-tropics (e.g. Munoz et al. 2003, Laube and Zotz 2006) it seems that there are many more common and intermediate epiphytic colonists, and less of a sharp distinction between dominant and less common species that is often seen in endophytic communities.

One way to visualize endophytic communities is to use rank abundance plots or other types of species abundance distributions (SADs) (McGill et al. 2007, Magurran 2004), which depict community structure in two-dimensional graphs. For example, He et

al. (2012), and Thomas and Shattock (1986) fit phyllosphere data using Preston's octave method. Thomas and Shattock (1986) also, and later Zambell and White (2014), plotted phyllosphere data using rank abundance plots. Rank abundance plots are very good for comparison of communities. In these plots, each species' abundance can be plotted on the y-axis, while species rank is plotted on the x-axis. The use of proportional abundance makes comparison easier in the face of different levels of sampling or very different total abundances, as plots can be fit to a similar scale on the y-axis. The common practice of putting the y-axis on a log scale (Magurran 2004), unless necessary to visualize very widely scattered data points, is probably ill advised as it is intuitively less meaningful and distorts perception.

Species abundance distributions can also be fit to different mathematical distributions, which is meant either as a way to identify universal trends, or a way to identify how different biological processes might be linked to certain patterns (Magurran 2004). Thomas and Shattock (1985), for instance, fit their phyllosphere dataset to both Fisher's logarithmic series, and to Motomura's geometric series. However, the current consensus is that fitting of patterns to a mathematical distribution does not prove any particular biological assembly process (McGill et al. 2007). Regardless, it is still useful to be familiar with the patterns as broad descriptions of plots, and SADs are still often used to generate evidence for different community assembly processes. Since interpretation of mathematical fittings is ambiguous, McGill et al. (2007) advised that a more fruitful use of SADs is in the search for empirical patterns along environmental gradients, successional or temporal processes, and in subsets of the main dataset. An example of gradients is the change in the structure of tree communities along increasing productivity

gradients (low to high altitude; high to low latitude) from a more geometric appearing curve (high dominance, few species) to a lognormal (more even and species rich) (Whittaker 1965, Hubbell 1979, described in McGill et al. 2007). Endophytic communities have not been examined for change along such gradients of elevation or latitude, though evidence suggests that for latitude at least, there are profound differences in community structure (Arnold and Lutzoni 2007).

Zambell and White (2014) used rank abundance plots to subset the phyllosphere community, by plotting endophytic, epiphytic, and combined culturable fungal communities of common greenbrier (*Smilax rotundifolia*) stems. This demonstrated that epiphytic communities showed less of a steep drop from dominant to rare species, as well as containing more species richness. Other potentially useful applications of rank abundance curves could include the sub-setting and recombining of communities in different plant tissues (see Chapter IV), different plant species, and different seasons throughout the year, as well as examination of successional change throughout seasons, in differently aged tissues, and in living to dying plant tissues. Since many latent endophytes may compete at the time of senescence, I predict that endophytic communities would take on an even stronger dominance structure at this time, as certain species most fit for the tissue type and weather conditions would out compete less adapted colonists. This is suggested by Douanla-Meli et al.'s (2013) finding that fungal diversity decreased in yellowing leaves of a *Citrus* species compared to healthy leaves. Rank abundance curves are also useful for comparison of large numbers of datasets. Differences between fungal endophyte or epiphyte communities and other types of communities (e.g. insect herbivores, vascular epiphytes, fungal rhizophere, bacterial

communities, mycorrhizal communities) might become apparent by comparison of many datasets, as well as differences in endophytes of different tissues, different biomes, different growth habits (forbs, shrubs, trees), clonal vs. scattered rare plants, or any other comparison for which there is adequate data.

Evolutionary patterns in endophytic assemblages

Evolutionary relationships among phyllosphere species can also be very informative. Several studies have provided clues as to evolutionary relationships between parasitic and endophytic isolates in the same genus. For example, the study of Ganley et al. (2004) of endophytes vs. pathogens of the same host, showed that endophytes while separate species from pathogens, were not in entirely separate monophyletic lineages. A multi-locus study of *Phyllosticta* endophytic and parasitic isolates also showed that endophytes and parasites were interspersed, not forming separate clades (Glienke et al. 2011). Phylogenetic studies have also indentified endophytic generalists. Glienke et al. (2011) found that *Phyllosticta capitalensis* infects many diverse hosts. Similarly, Rojas et al. (2010), in a multi-locus study, found that a species of *Colletotrichum, C. tropicale,* infected many hosts. A multi-locus approach was used by Gazis et al. (2011) to study endophytic isolates of *Colletotrichum*, *Trichoderma*, and *Pestalotiopsis*, and it was demonstrated that ITS underestimated the number of species involved. Moving forward, it is important that more than one gene be used to characterize endophytic evolutionary relationships.

Another approach that may be applied to endophytes are studies of phylogenetic community structure (also discussed by Saunders and Kohn 2010), a type of research that

focuses on evolutionary relationships between co-occurring species within the same community. These types of studies are typically focused on a particular genus or family in which multiple species occur throughout a region. The first such studies were species per genus ratio studies, which asked, within a particular place or habitat, if there were more or less species per genus than expected by chance (Elton 1946, Simberloff 1970). It was expected that species which were too similar in niche requirements could not co-exist, while conversely, in some situations certain adaptations might make many species within the same genus adapted to a type of habitat. This concept has been further developed, incorporating phylogenetic data and in some studies the relationship to phenotypic traits (Webb 2000, Webb et al. 2002, Cavender-Barres et al. 2004, Cavender-Barres et al. 2006). These approaches formally test for non-random patterns in which co-occurring species are more or less related to each other than expected by chance, with null models derived from a regional species pool (for review, see Vamosi et al. 2009).

The study of fungal endophytes presents some challenges in this type of research. First, use of the ITS gene alone could obscure our ability to see that very closely related species might share similar niches, entirely defeating the purpose of this type of analysis. Suitable genes will have to be chosen based on which fungal taxon group is targeted for study. Further, the use of morphology beyond the genus level, to sort different species, could be problematic as it might result in lumping of indistinguishably similar species (Rojas et al. 2010); so it would be ideal to sequence genes of many isolates. Finally, the choice of a species pool could be problematic, since many micro-fungi are undescribed, and invisible. It will probably be necessary to create a null model of a species pool, by sampling multiple species of co-occurring plants. Thus the species pool, and its

phylogeny cannot be downloaded, but will have to be created directly by the researcher. Very speciose endophytic genera would be best for generating effective null models with many branches. For example, Chareprasert et al. (2005) reported that teak leaves contained 8 *Phomopsis* morphotypes and 11 *Xylaria* morphotypes, while rain tree yielded 7 *Phomopsis* morphotypes and no *Xylaria* isolates. Detection of phylogenetic cluster and dispersion are also highly dependent on the scale of study (Swenson et al. 2006). Depending on the analysis and designation of what constitutes a sample, different patterns may emerge. The analysis of different subsets of a dataset at the level of single vs. multiple plant species, genera, tissue types, or sites could be very informative. Another intriguing question exists: how would phylogenetic structure change from living to dead tissues? Environmental filtering may select for a clustered community in living plants, while competitive exclusion creates an over-dispersed community in dead tissue. It is also likely, given the reported frequency of generalist species in the tropics (e.g. Okane et al. 2011), that patterns will differ between temperate and tropical regions.

In my studies, while I did not conduct phylogenetic community analysis, I did use backbone phylogenies of described species to infer where endophytic strains fell in the phylogeny, and to get some idea of how commonly occurring endophytes in the same plant, and the same fungal genus, were related. *Smilax rotundifolia* is commonly colonized by 2 *Colletotrichum* spp., 2 *Phyllosticta* spp., and 3-4 common *Pestalotiopsis* spp. Using reference phylogenies of described species in these groups, reveals that none of the co-dominant congeners is closely related to each other, but appear scattered widely throughout the known phylogeny of the genus. As no phyllosphere species were sister groups, it can be concluded that there was no speciation of a single symbiote into

multiple within the same host, but rather, host shifts between fungi of different plants are the likely scenario. These widely separated species in the phylogenies may have different traits that allow them to occupy different niches in the same plant.

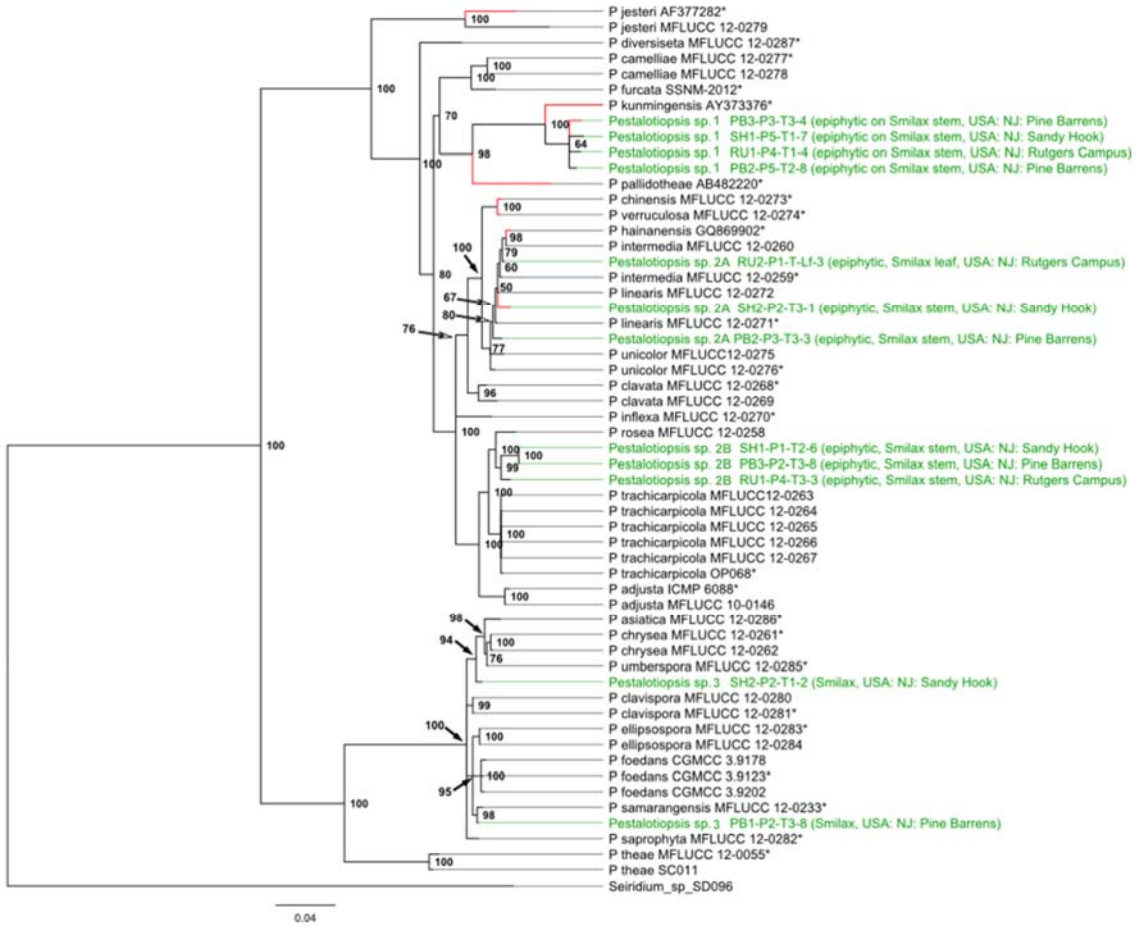

Fig. V-1. Evolutionary relationships between *Pestalotiopsis* epiphytes of greenbrier

Phylogenetic tree based on Bayesian analysis of a partitioned alignment of genes ITS (HKY+G model) and Tef1 (GTR+I+G model) including my own isolates (indicated in green) and sequences downloaded from Genbank according to the accession numbers given in the dataset of Maharachchikumbura et al. (2012). Red branches are attached to species for which only the ITS gene was available. Asterisks indicate ex-type or ex-epitype cultures. Posterior probabilities are shown at the nodes.

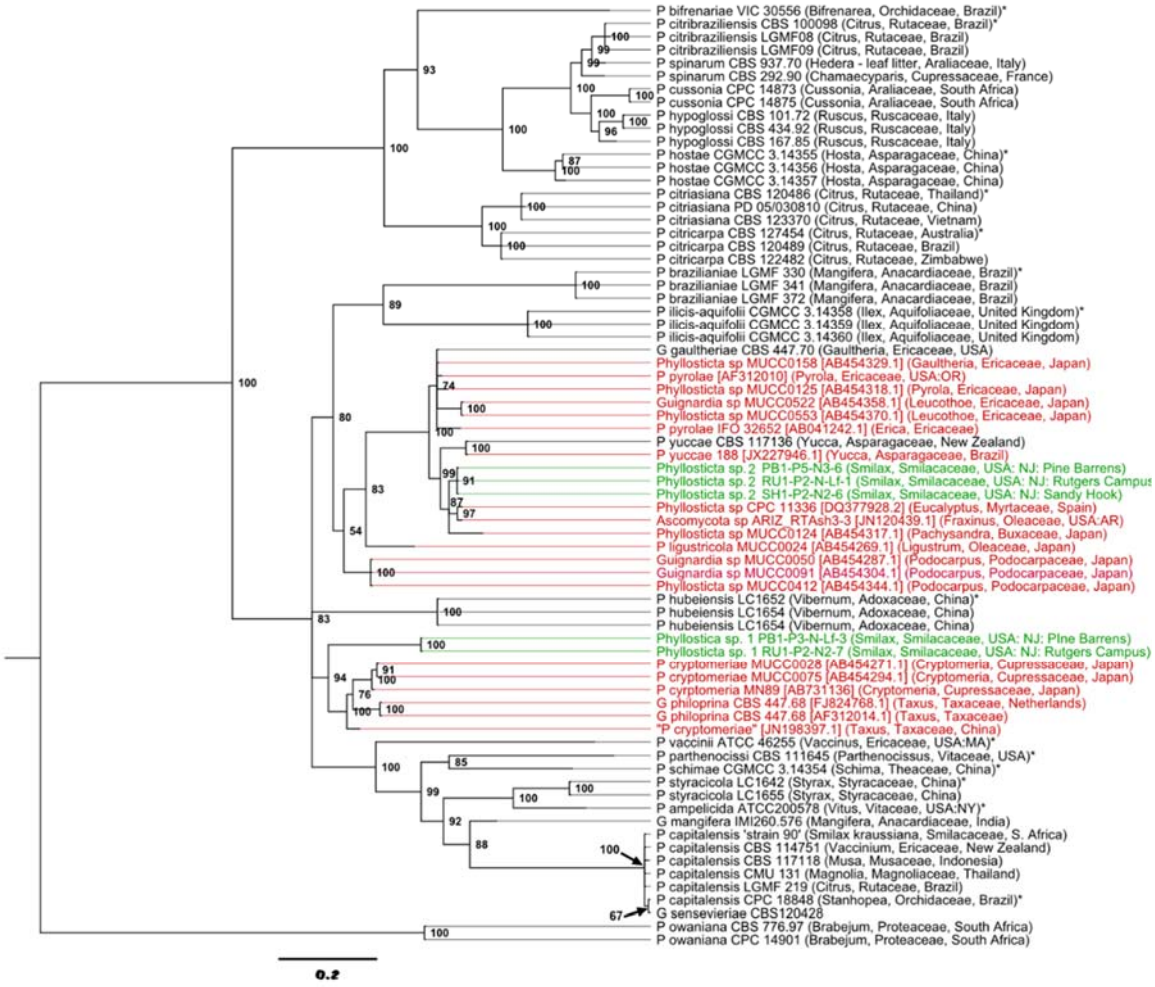

Fig. V-2. Evolutionary relationship between *Phyllosticta* endophytes of greenbrier

Phylogenetic tree based on Bayesian analysis of a partitioned alignment of genes ITS (SYM+I+G model) and Tef1 (HKY+G model) including my own sequences (indicated in green) combined with Genbank sequences that were downloaded according to accession numbers given in Glienke et al. (2011), Su and Cai (2012) and Zhang et al. (2013a, 2013b). Also included are the top 10 Blast search ITS results (shown in red) after Blast searching one of each of my *Phyllosticta* morphotypes. Asterisks indicate ex-type, ex-epitype, or ex-neotype cultures.

6. Understanding the role of secondary metabolites on endophytic colonization

One of the major sources of variability in the internal plant environment is undoubtedly the secondary metabolites contained there. Plant secondary metabolite structures and pathways have diversified over different evolutionary lineages in the plant

kingdom, so that each plant is often dominated by a particular chemistry, but with variations between different tissues, organs, populations, and developmental stages even within a single plant species (Wink 2003). Research into the influence of plant secondary metabolites on endophytic fungi has been highly suggestive so far, justifying more detailed studies in the future. Sanchez-Azofeifa et al. (2012) found correlations between endophytic species richness and several variables including water content, and total chlorophyll, and the ratio of polyphenols to specific leaf weight. Bailey et al. (2005) found a negative correlation between infection frequency of total endophytes in poplar (*Populus* spp.) twig bark and concentration of condensed tannins, but did not find relationships to total phenolic glycosides, or to two specific phenolic glycosides (Salicortin & HCH-salicortin). Shubin et al. (2014) studied the relationship of endophytic communities in rhizomes of the medicinal monocot *Alpinia officinarum* to both total volatile oils and the flavonol galangin. Using a culture-independent approach (clone libraries combined with T-RFLP profiling), and cluster analysis, they showed that endophytic communities formed four clusters that corresponded to four levels of active chemicals (low, intermediate, sub-high, and high; total volatiles and galangin were positively correlated). Saunders and Kohn (2009) showed that maize plants producing BX toxins were colonized by a higher proportion of BOA tolerant fungi (established in assays on toxin supplemented growth medium), and were more frequently infected by *Fusarium* isolates.

More detailed studies of plant chemistry and endophytes, with emphasis on individual endophytic species, and a breakdown of specific secondary metabolites by tissue, might greatly advance our understanding of plant-fungal relationships. Thousands

of plant-produced chemical compounds have been described, which in general are hypothesized to function as *in planta* antibiotics and/or play a role in signaling (Piasecka et al. 2015). For example, Osbourn (1996) lists as fungitoxic compounds "phenols and phenolic glycosides, unsaturated lactones, sulfur compounds, saponins, cyanogenic glycosides, glucosinolates...resorcinols and dienes." Hoffland et al. (1996) provided indirect evidence for the importance of constitutive chemical defense for fungal resistance of radish plants. They tested 13 radish cultivars for susceptibility to a single pathogenic strain of *Fusarium oxysporum*, as well as for their relative growth rate in the absence of the pathogen. A negative correlation was found between growth rate and resistance, explaining 68% of the variation in resistance. Chemical analysis showed that the roots of slower growing cultivars accumulated more protein and more phenols, possibly representing soluble phenols, or parts of lignin or proteins. From this evidence, Hoffland et al. (1996) suggested that slower growing cultivars allot more energy to constitutive chemical defense, and are in turn more resistant.

Exposure to secondary metabolites in phyllosphere fungi depends on each species' mode of colonization (e.g. intercellular, intracellular, substomatal, or epiphytic), as well as the packaging of secondary metabolites by the plant, and whether they are diffusible to apoplastic fluids and surfaces, or sealed within organelles (Osbourn 1996). Saunders and Kohn's (2010) description of plant compounds as "environmental filters" is a useful way to think of the different layers of defense as fungi attempt to utilize the plant habitat. Indole glucosinolates (IGs) and BXs are chemicals implicated in pre-invasive, surface inhibition of pathogens (Piasecka et al. 2015). Endophytic colonists that survive surface chemistry, might, after penetrating a plant, experience additional compounds diffused in

the apoplast. Such colonists may trigger the release compounds stored in vacuoles, acting as a second round of filters on colonization success.

If an endophyte is able to survive the surface invasion, and internal establishment, there is further evidence that secondary metabolites may be able to exert an influence on latent endophytic infections once established. This comes from the literature of post-harvest pathogens of fruits: these are fungi that have a latency period before attacking ripening fruit. Some of these only form surface appresoria, while others are endophytic in that they colonize internal tissue, then lie quiescent. Prusky (1996) has reviewed the evidence that preformed anti-fungal compounds in fruit serve as inhibitors to fungal attack, and that their sharp decrease in ripening fruit may trigger termination of quiescence and necrotrophic activity in structures such as appresoria and infection pegs. Examples include (1) tomatine, a saponin found in green tomatoes, which may inhibit quiescent *Botrytis cinerea*, (2) derivatives of the phenolic compound resorcinol in skin of mangos as inhibitors of *Alternaria alternata*, (3) diene and monoene compounds in avocado peels as inhibitors of *Colletotrichum gloeosporioides*, and (4) the monoterpene aldehyde citral in lemons may also be involved in post-harvest decay resistance to *Penicillium* (in this last case no latency period is necessarily involved). There is also some evidence that absence of inducible anti-fungal compounds, phytoalexins, in some ripe fruits may be linked to awakening of quiescent fungi (Prusky 1996, 2013).

Recent findings involving a phytoalexin called camalexin, an indole alkaloid produced by *Arabidopsis*, show how the loss of phytoalexin production may allow latent infections to commence growth. A mutant strain of *Arabidobsis* exists that is unable to

produce camalexin. Experiments have shown that the fungus *C. gloeosporioides* was able to penetrate plants whether they produced camalexin or not, but necrotrophic expansion of the fungus was halted only in plants that could produce the phytoalexin (Piasecka et al. 2015).

If quiescent pathogens in fruit are able to detect a reduction in the level of fungitoxic compounds as fruit ripens, then we can infer that some phytochemicals exert a continuous activity on quiescent fungal infections beyond just the period of initial colonization. If constituent chemical compounds can cause a fungal infection to remain quiescent, then they might also, at a higher concentration, or given enough time, cause the death of an infective mycelium.

In keeping with our objective to be mindful of the entire endophytic life-cycle, it might be useful to think of chemical exposure in three main phases: (1) colonization, including exposure to surface compounds, apoplast compounds, compounds released from vacuoles by tissue destruction or plant responses, and phytoalexins produced immediately upon infection; (2) latent residency, involving long term exposure to constituent plant chemistry, or exposure to phytoalexins produced some time after the endophyte colonized (e.g. in response to another pathogen); and, (3) growth phase at tissue or plant death, involving exposure to residual plant compounds that are no longer being actively produced in senescing or dead plant tissue.

The studies of Espinosa-Garcia et al. (1996) support a view of endophytes as behaving similar to post-harvest pathogens as outlined above. Espinosa-Garcia et al. (1996) performed a series of experiments in which they examined the relationship

between endophytes sampled from redwoods, and mixtures of volatile terpenes that reflected different redwood phenotypes. They expected that the endophytes would show higher tolerance to the particular host phenotype from which they were isolated—a pattern that has been observed in conifer pathogens. Instead they found that 13 isolates of a common redwood endophyte, *Pleuroplaconema* sp., showed low average tolerance, low variability between different combinations, and showed no evidence of special adaptation to the host phenotype they were taken from (Espinosa-Garcia and Langenheim 1991). Since tolerance was poor, they hypothesized that essential oils may limit the growth of endophytes, and that reduction in concentration may allow for endophytic breaking of quiescence. This hypothesis was supported by in vitro experiments. Two redwood endophytes were shown to be uninhibited or even stimulated in their growth at low terpene concentrations, while at high concentrations growth they were inhibited. In another study, they found that under exposure to the redwood terpenes the douglas fir endophyte, *Rhabdocline parkeri*, was the most heavily inhibited compared to three endophytic isolates of redwood and a fungal generalist pathogen. In summary, the analysis of Espinosa-Garcia et al. (1996) suggest that plant chemistry may be least inhibitory to virulent pathogens, moderately inhibitory to host adapted endophytes, and most inhibitory to endophytes of other hosts. This also raises the question of what would happen if an endophytic strain becomes better adapted for growth in the face of host chemistry? Perhaps those mutants with too great a tolerance prematurely enter a saprophytic or necrotrophic state, and are killed by the host response. On the other hand, tolerant endophytes may become successful pathogens.

While some studies have linked specific chemicals to influencing endophytes, no studies have conclusively linked the recurring niche related patterns seen in endophytic communities to plant chemistry, patterns including genus or species level host preference, tissue preference, tissue age, and high dominance of single species. Of the niche axes identified in endophytes, the majority could conceivably be linked to changes in plant chemistry that correlate to the factor being studied. Changes in plant chemistry have been demonstrated along base-to-tip axes of plant-tissues (Fischer et al. 2011, Rohloff 1999), between different tissue types (Rohloff 1999), as tissue ages (Coley and Barone 1996) and between different seasons (Hussain et al. 2008). More detailed studies moving beyond single chemicals, and beyond broad conclusions of infection frequency, to the influence of individual chemical constituents on individual fungi might lead to fresh insights in both the study of plant phytochemical diversity and endophytology.

Gradients can be particularly useful for correlative purposes, in demonstrating variables that influence communities. The key is to find those niche axes that vary continuously. For example, different tissue are discrete categories. On the other hand, chemistry and seasonal change can be measured continuously. The continuous base to tip tissue gradients are probably one of the most promising areas of study, with less co-variants to worry about than season or tissue age (e.g. chemistry might change seasonally, but also temperature, humidity, tissue age, toughness, inoculum density).

By sampling 1-cm segments of stem from the vine *Smilax rotundifolia* at 15 cm intervals from the base and the tip of the plants, I found that isolates of a *Colletotrichum* sp., a *Phomopsis* sp., and an *Aureobasidium* sp. showed strong preference for different

heights along the stem (Ch. III; Zambell and White 2014). In this study, each 1-cm segment was subdivided into 12 fragments, allowing a strong quantitative assessment of species affinity for each stem position, and the ability to see continuous trends in the consecutive positions. A similar trend, in a leaf rather than a stem, can also be seen in a study by Hata and Futai (1995), in which needles of *Pinus densiflora* and a hybrid *Pinus* were divided into 8 segments running from tip to base. The most basal segment was dominated by *Phialocephala* sp., which appeared to become less common in the consecutively higher samples, being absent in the last 2-3 closest to the tip. *Leptostroma* spp. (anamorph of *Lophodermium*) on the other hand, were common in the segments near the tip, and decreased to zero presence in the most basal segment. This trend was visible by using a simple measure of presence counts per segments sampled. If each segment was subdivided, or DNA of different species quantified, an even stronger trend might emerge. In a later study, Hata and Futai (1996) showed there is likely a similar tip to base gradient of colonization preference in other species—though in this case they did not dissect the entire needle, but only sampled a basal and a middle segment across many *Pinus* spp. in an arboretum. *Phialocephala* again dominated basal segments in many species, and *Leptostroma* spp. again were common in the middle segment, along with *Cenangium ferruginosum* in some species. Gradients like this, especially in conifer needles, may not be uncommon, as some of the early seminal studies in endophytology have demonstrated or commented that the most basal segments of *Pinus* or other conifer needles show restriction or dominance patterns between different endophytes between the basal, or "petiole" segment, and the middle, or "blade," segment. (Bernstein and Carroll 1977, Carroll et al. 1977, Carroll and Carroll 1978). These early studies did not study this

phenomenon as a gradient however, plotting each position graphically, but as two discrete states. Carroll and Petrini (1983) showed evidence that fungi from petiole vs. blade segments could be adapted to digestion of different substrate components reflecting their habitat. This however, related to post senescent consumption of the needle, and does not explain how they arrived in their position as latent infections. The *Pinus* needle system, although one of the oldest studied, may be the best place to begin to study what causes endophytic niche partitioning along a tissue gradient.

7. Summary

In summary, I have hopefully identified some useful new ways to study endophytic community structure and assembly. Many unanswered questions about endophytic community formation have been raised throughout this chapter. To recap, these are: (1) Can we measure, at least partially, the dynamics of endophytic community assembly in terms of colonization rate and mortality rates of colonies? (2) What happens when an endophytic species dominates living tissue as it transitions into the more competitive environment of dying or dead tissue, and is the outcome variable depending on mode of senescence, seasons or other factors? (3) What other transitions take place in endophytic communities between living, weakened, and dead tissue in terms of species abundance patterns (rank abundance plots, richness, evenness, diversity), and phylogenetic community structure? (4) Do non-random phylogenetic patterns exist in endophytic communities? (5) Are endophytes that form local infections, and that do not have a surface presence, able to compete in living tissues, and if so, how? (6) Can Class III endophytes, forming localized infections, in natural systems, change host chemistry by their presence? (7) Is host chemistry connected to the niche partitioning observed along

many axes including host, tissue, position from base to tip of a single plant organ, and season? (8) Mechanistically, if host chemistry does influence selection of species in the endophytic community, then is this because less fit endophytic propagules fail to survive the host surface, fail to penetrate the host, fail to establish infections, fail to maintain living quiescent infections because of the chemical environment, or fail to maintain quiescence thereby triggering a plant response that kills them? Which of these mechanisms might operate in selecting for fungi that are co-dominant but subtly adapted to different tissues, tissue ages, or other variables?

Answering these questions will have application beyond simply understanding endophytic communities for their own sake. The desire to alter the fungal community might be facilitated by a stronger knowledge of endophytic community assembly, and future experiments should also be directed at understanding how malleable phyllosphere communities are by human intervention. Also, understanding endophytes might give us insight into pathogens as well, as some endophytes exist on the continuum of pathogenicity and are closely related to pathogens. For example, the endophytic pathogen, *Phomopsis viticola* (Mostert et al. 2000), was constrained to nodes and internodes in *Vitis* vines. Understanding why endophytes are tissue specific could be useful in the prevention of plant disease. Finally, whether endophytes are tightly coupled to host or not, their potential for parasitism may be a major driver for plant evolution, in the maintenance and diversification of plant secondary metabolites over evolutionary time.

BIBLIOGRAPHY

Chapter 1

Ai, D., Desjardins-Proulx P., Chu C., Wang, G. (2012). Immigration, local dispersal limitation, and the repeatability of community composition under neutral and niche dynamics. *PLoS ONE* 7: e46164.

Chave, J., Muller-Landau, H.C., Levin, S.A. (2002). Comparing classical community models: theoretical consequences for patterns of diversity. *American Naturalist* 159:1-23.

Von Humboldt, A., Bonpland, A. (2009). *Essay on the Geography of Plants*, *Edited with an Introduction by Stephen T. Jackson, Translated by Sylvie Romanovski*. Chicago, University of Chicago Press, 274 pp.

Chapter 2

Allen, R.L., Bittner-Eddy, P.D., Grenville-Briggs, L.J.,Meitz, J.C., Rehmany, A.P., Rose, L.E., Beynon, J.L. (2004). Host-parasite coevolutionary conflict between *Arabidopsis* and downey mildew. Science 306:1957-1960.

Cameron , K.M., Fu, C. (2006). A nuclear rDNA phylogeny of *Smilax* (Smilacaceae). *Aliso* 22:598-605.

Coker, W.C. (1944). The woody smilaxes of the United States. *Journal of the Elisha Mitchell Scientific Society* 60:27-69.

Gleason, H.A., Cronquist, A. (1991). *Manual of Vascular Plants of Northeastern United States and Adjacent Canada,* 2nd ed. Bronx, New York, The New York Botanical Garden, 910 pp.

Goodrum, P.D. (1977). Greenbriers/Smilax spp. In *Southern Fruit Producing Woody Plants Used by Wildlife, Gen. Tech. Rep. SO-16,* L. K. Halls, Ed. New Orleans, LA, U.S. Department of Agriculture, Forest Service, Southern Region, Southern Forest Experiment Station, pp. 110-116.

Holmes, W.C. (2002). Smilax. In *Flora of North America North of Mexico, Vol. 26*, e. Flora of North America Editorial Committee, Eds. New York and Oxford, page online {http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=130567}). New York and Oxford.

Landers, L.J., Hamilton, R.J., Johnson, A.S., Marchinton, R.L. (1979). Foods and habitat of black bears in southeastern North Carolina. *The Journal of Wildlife Management* 43:143-153.

McEwan, R.W., Paratley, R.D., Muller, R.N., Riccardi, C.L. (2005). The vascular flora of an old-growth mixed mesophytic forest in southeastern Kentucky. *The Journal of the Torrey Botanical Society* 132:618-627.

Ohman, M.C. (2006). *Characteristics of Fuel Beds Invaded by Smilax rotundifolia.* Masters Thesis, University of Massachusetts Amherst, 57 pp.

Qi, Z., Cameron, K.M., Li, P., Zhao, Y., Chen, S., Chen, G., Fu, C. (2013). Phylogenetics, character evolution, and distribution patterns of the greenbriers, Smilacaceae (Liliales), a near-cosmopolitan family of monocots. *Botanical Journal of the Linnean Society* 173:535–548.

Rhoads, A.F., Block, T.A. (2007). *The Plants of Pennsylvania,* 2nd ed. Philadelphia, University of Pennsylvania Press, 1042 pp.

Smith, R.L. (1974). Greenbriers. In *Shrubs and Vines for Northeastern Wildlife. General Technical Report NE-9,* Gill, J.D. Healy, W.M., Eds. Upper Darby, PA, U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station, pp. 54-58.

USDA, NRCS (2015). The PLANTS Database (http://plants.usda.gov, 14 May 2015). National Plant Data Team, Greensboro, NC 27401-4901 USA.

Chapter 3

Adler, P.B., HilleRisLambers, J., Levine, J. M. (2007). A niche for neutrality. *Ecology Letters* 10:95-104.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25:3389-3402.

Amarasekare, P. (2003). Competitive coexistence in spatially structured environments: a synthesis. *Ecology Letters* 6:1109-1122.

Ando, K. (1992). A study of terrestrial aquatic hypohomycetes. *Transactions of the Mycological Society of Japan* 33:415-425.

Arnold, A.E., Herre, E.A. (2003). Canopy cover and leaf age affect colonization by tropical fungal endophytes: ecological pattern and process in *Theobroma cacao* (Malvaceae). *Mycologia* 95:388-398.

Arnold, A.E., Lutzoni, F. (2007). Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? *Ecology* 88: 541-549.

Balint, M., Tiffin, P., Hallstrom, B., O'Hara, R.B., Olson, M.S., Frankhauser, J.D., Piepenbring, M., Schmitt, I. (2013). Host genotype shapes the foliar fungal microbime of balsam poplar *(Populus balsamifera). PLoS One* 8:e53987.

Bernstein, M.E., Carroll, G.C. (1977). Internal fungi in old-growth Douglas fir foliage. *Canadian Journal of Botany* 55:644-653.

Bilgrami, K.S. (1963). Studies on conidial dispersal of some pathogenic species of *Phyllosticta. Naturwissenschaften* 50:360.

Boyle, C., Gotz, M., Dammann-Tugend, U., Schulz, B. (2001). Endophyte-host interactions III. Local vs. systemic colonization. *Symbiosis* 31:259-281.

Bray, J.R., Curtis, J.T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs* 27:325-349.

Cabral, D. (1985). Phyllosphere of *Eucalyptus viminalis:* dynamics of fungal populations. *Transactions of the British Mycological Society* 85:501-511.

Carnegie, S.F. (1980). Aerial dispersal of the potato gangrene pathogen, *Phoma exigua* var. *foveata. Annals of Applied Biology* 94:165-173.

Carroll F.E., Muller E., Sutton B.C. (1977). Preliminary studies on the incidence of needle endophytes in some European conifers. *Sydowia* 29:87-103.

Challinor, V.L., Parsons, P.G., Chap, S., White, E.F., Blanchfield, J.T., Lehmann, R.P., De Voss, J.J. (2012). Steroidal saponins from the roots of *Smilax* sp.: structure and bioactivity. *Steroids* 77:504-511.

Chao, A. (1984). Non-parametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics* 11:265-270.

Chao, A. (1987). Estimating the population size for capture-recapture data with unequal catchability. *Biometrics* 43:783-791.

Chave, J., Muller-Landau, H.C., Levin, S.A. (2002). Comparing classical community models: theoretical consequences for patterns of diversity. *The American Naturalist* 159:1-23.

Clarke, K.R. (1993). Non-parametric multivariate analysis of changes in community structure. *Australian Journal of Ecology* 18:117-143.

Colwell, R.K. (2013). EstimateS: statistical estimation of species richness and shared species from samples. Version 9. Persistent URL <http:// purl.oclc.org/estimates>.

Colwell, R.K., Mao, C.X., Chang, J. (2004). Interpolating, extrapolating, and comparing incidence-based species richness. *Ecology* 85:27172727.

Colwell, R.K., Rangel, T.F. (2009). Hutchinson's duality: the once and future niche. *Proceedings of the National Academy of Sciences of the United States of America* 106:19651-19658.

Cordier, T., Robin, C., Capdevielle, X., Desprez-Loustau, M.L., Vacher, C. (2012). Spatial variability of phyllosphere fungal assemblages: genetic distance predominates over gographic distance in a European beech stand *(Fagus sylvatica). Fungal Ecology* 5:509-520.

Damm, U., Verkley, G.J.M., Crous, P.W., Fourie, P.H., Haegi, A., Riccioni, L. (2008). Novel *Paraconiothyrium* species on stone fruit trees and other woody hosts. *Persoonia* 20:9-17.

Davey, M.L., Heegaard, E., Halvorsen, R., Ohlson, M., Kauserud, H. (2012). Seasonal trends in biomass and structure of bryophyte-associated fungal communities explored by 454 pyrosequencing. *New Phytologist* 195:844-856.

Devarajan, P.T., Suryanarayanan, T.S. (2006). Evidence for the role of phytophagous insects in dispersal of non-grass fungal endophytes. *Fungal Diversity* 23:111-119.

Dickinson, C.H. (1965). The mycoflora associated with *Halimione portulacoides:* III. Fungi on green and moribund leaves. *Transactions of the British Mycological Society* 48:603-610.

Elamo, P., Helander, M.L., Saloniemi, I., Neuvonen, S. (1999). Birch family and environmental conditions affect endophytic fungi in leaves. *Oecologia* 118:151-156.

Elton, C. (1927). *Animal Ecology*. New York, The Macmillan Company, 207 pp.

Espinosa-Garcia, F.J., Rollinger, J.L., Langenheim, J.H. (1996). Coastal redwood endophytes: their occurrence, interactions and response to host volatile terpenoids. In Endophytic fungi in grasses and woody plants, Redlin, S.C., Carris, L.M., Eds. St. Paul, APS Press, pp. 87-100.

Fischer, R., Nadav, N., Chaimovitsh, D., Rubin, B., Dudai, N. (2011). Variation in essential oil composition within individual leaves of sweet basil *(Ocimum basilicum* L.) is more affected by leaf position than by leaf age. *Journal of Agricultural and Food Chemistry* 59:4913-4922.

Fisher, P.J., Anson, A.E., Petrini, O. (1986). Fungal endophytes in *Ulex europaeus* and *Ulex gallii. Transactions of the British Mycological Society* 86:153-193.

Frohlich, J., Hyde, K.D., (1999). Biodiversity of palm fungi in the tropics: are global fungal diversity estimates realistic? *Biodiversity and Conservation* 8: 977-1004.

Flessa, F., Persoh, D., Rambold, G. (2012). Annuality of central European deciduous tree leaves delimits community development of epifoliar pigmented fungi. *Fungal Ecology* 5:554-561.

Gao, X.X., Zhou, H., Xu, D.Y., Yu, C.H., Chen, Y.Q., Qu, L.H. (2005). High diversity of endophytic fungi from the pharmaceutical plant, *Heterosmilax japonica* Kunth revealed by cultivation-independent approach. *FEMS Microbiology Letters* 249:255-266.

Gazis, R., Chaverri, P. (2010). Diversity of fungal endophytes in leaves and stems of wild rubber trees *(Hevea brasiliensis)* in Peru. *Fungal Ecology* 3:240-254.

Geange, S., Pledger, S., Burns, K.C., Shima, J.S. (2011). A unified analysis of niche overlap incorporating data of different types. *Methods in Ecology and Evolution* 2:175-184.

Gause, G.F. (1934). *The struggle for existence*. Baltimore, Williams and Wilkins, 192 pp.

Gonczol, J., Revay, A. (2004). Fungal spores in rainwater: stemflow, throughfall and gutter conidial assemblages. *Fungal Diversity* 16:67-86.

Gonczol, J., Revay, A. (2006). Species diversity of rainborne hyphomycete conidia from living trees. *Fungal Diversity* 22:37-54.

Hammer, O., Harper, D.A.T., Ryan, P.D. (2001). PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4(1):9 pp.

Hudson, H.J. (1968). The ecology of fungi on plant remains above the soil. *New Phytologist* 67:837-874.

Hudson, H.J., Webster, J. (1958). Succession of fungi on decaying stems of *Agropyron repens. Transactions of the British Mycological Society* 41:165-177.

Hutchinson, G.E. (1957). Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology* 22:415-427.

Hutchinson, G.E. (1978). *An Introduction to Population Ecology*. New Haven, Yale University Press, 260 pp.

Jost, L. (2006). Entropy and diversity. *Oikos* 113:363-375.

Jumpponen, A., Jones, K.L. (2010). Seasonally dynamic fungal communities in the *Quercus macrocarpa* phyllosphere differ between urban and nonurban environments. *New Phytologist* 186:496-513.

Kendrick, B., Burges, A. (1962). Biological aspects of the decay of *Pinus sylvestris* leaf litter. *Nova Hedwigia* 4:313-342.

Kerr, B., Riley, M.A., Feldman, M.W., Bohannan, B.J.M. (2002). Local dispersal promotes biodiversity in a real-life game of rock-paper-scissors. *Nature* 418:171-174.

Kharwar, R.N., Gond, S.K., Anuj, K., Mishra, A. (2010). A comparative study of endophytic and epiphytic fungal association with leaf of *Eucalyptus citriodora* Hook., and their antimicrobial activity. *World Journal of Microbiology and Biotechnology* 26:1941-1948.

Koljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglass, B., Drenkhan, T., Eberhardt, U., Duenas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lucking R., Martin M.P., Matheny P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Poldmaa, K., Saag, L., Saar, I., Schubler, A., Scott, C.S., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.-H. (2013). Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* 22: 5271-5277.

Legault, D., Dessureault, M., Laflamme, G. (1989a). Mycoflore des aiguilles de *Pinus banksiana* et *Pinus resinosa* I. champignons endophytes. *Canadian Journal of Botany* 67:2052-2060

Legault D., Dessureault M., Laflamme, G. (1989b). Mycoflora of *Pinus banksiana* and *Pinus resinosa* needles. II. Epiphytic fungi. *Canadian Journal of Botany* 67:2061-2065.

Luttrell, E.S. (1944). The morphology of *Myiocopron smilacis* (De Not.) Sacc. *American Journal of Botany* 31:640-649.

Magurran, A.E. (2004). *Measuring Biological Diversity*. Malden, Blackwell Science Ltd, 256 pp.

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research* 27:209-220.

Macauley, B.J., Thrower, L.B. (1966). Succession of fungi in leaf litter of *Eucalyptus regnans. Transactions of the British Mycological Society* 49:509-520.

Mariano, R.L.R., Lira, R.V.F., Silveira, E.B., Menezes, M. (1997). Levantamento de fungos endofiticos e epifiticos em folhas de coqueiro no Nordeste do Brasil. I. Frequuencia da populapao fungica e efeito da hospedeira. *Agrotropica* 9:127-134.

Marquez, S.S., Bills, G.F., Zabalgogeazcoa, I. (2007). The endophytic mycobiota of the grass *Dactylis glomerata. Fungal Diversity* 27:171-195.

Mishra, A., Gond, S.K., Kumar, A., Sharma, V.K., Verma, S.K., Kharwar, R.N., Sieber, T.N. (2012). Season and tissue type affect fungal endophyte communities of the Indian medicinal plant *Tinospora cordifolia* more strongly than geographic location. *Microbial Ecology* 64:388-398.

Moncalvo, J.M., Wang, H.H., Hseu, R.S. (1995). Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacer and 25S ribosomal DNA sequences. *Mycologia* 87:223-238.

Moy, M., Belanger, F., Duncan, R., Freehoff, A., Leary, C., Meyer, W., Sullivan, R., White, J.F. (2000). Identification of epiphyllous mycelial nets on leaves of grasses infected by Clavicipitaceous endophytes. *Symbiosis* 28: 291-302.

Mucciarelli, M., Camusso, W., Maffei, M., Panicco, P., Bicchi, C. (2007). Volatile terpenoids of endophyte-free and infected peppermint *(Mentha piperita* L.): chemical partitioning of a symbiosis. *Microbial Ecology* 54:685-696.

Munafo, J.P., Gianfagna, T.J. (2011). Quantitative analysis of steroidal glycosides in different organs of Easter lily *(Lilium longiflorum* Thunb.) by LC-MS/MS. *Journal of Agricultural and Food Chemistry* 59:995-1004.

Nix-Stohr, S., Moshe, R., Dighton, J. (2008). Effects of propagule density and survival strategies on establishment and growth: further investigations in the phylloplane fungal model system. *Microbial Ecology* 55:38-44.

Osono, T. (2002). Phyllosphere fungi on leaf litter of *Fagus crenata:* occurrence, colonization, and succession. *Canadian Journal of Botany* 80:460-469.

Osono, T. (2008). Endophytic and epiphytic phyllosphere fungi of *Camellia japonica:* seasonal and leaf age-dependent variations. *Mycologia* 100:387-391.

Osono, T., Bhatta, B.K., Takeda, H. (2004). Phyllosphere fungi on living and decomposing leaves of giant dogwood. *Mycoscience* 45:35-41.

Osono, T., Mori, A. (2004). Distribution of fungi within the canopy of giant dogwood. *Mycoscience* 45:161-168.

Osono, T., Takeda, H. (1999). Decomposing ability of interior and surface fungal colonizers of beech leaves with reference to lignin decomposition. *European Journal of Soil Biology* 35:51-56.

Parker, P.E., Ramsdell, D.C. (1977). Epidemiology and chemical control of *Phomopsis* canker of highbush blueberry. *Phytopathology* 67:14811484.

Persoh, D. (2013). Factors shaping community structure of endophytic fungi-evidence from the Pinus-Viscum-system. *Fungal Diversity* 60: 55-69.

Petrini, O. (1991). Fungal endophytes of tree leaves. In *Microbial Ecology of Leaves,* Andrews, J.H., Hirano, S.S., Eds. New York, Springer, pp. 179-197.

Petrini, O. (1996). Ecological and physiological aspects of host-specificity in endophytic fungi. In *Endophytic Fungi in Grasses and Woody Plants.* Redlin, S.C., Carris, L.M., Eds. St. Paul, APS Press, pp. 87-100.

Prior, R., Gorges, K., Yurkov, A., Begerow, D. (2014). New isolation method for endophytes based on enzyme digestion. *Mycological Progress* 13:849-856.

Pugh, G.J.F., Buckley, N.G. (1971). The leaf surface as a substrate for colonization by fungi. In *Ecology of Leaf Surface Micro-Organisms*, Preece T.F., Dickinson C.H., Eds. London, Academic Press, pp. 431-445.

Qi, Z., Cameron, K.M., Li, P., Zhao, Y., Chen, S., Chen, G., Fu, C. (2013). Phylogenetics, character evolution, and distribution patterns of the greenbriers, Smilacaceae (Liliales), a near-cosmopolitan family of monocots. *Botanical Journal of the Linnean Society* 173:535-548.

Revay, A., Gonczol, J. (2011). Canopy fungi ("terrestrial aquatic hyphomy- cetes") from twigs of living evergreen and deciduous trees in Hungary. *Nova Hedwigia* 92:303-316.

Reynolds, D.R. (1999). *Capnodium citri:* the sooty mold comprising the taxon concept. *Mycopathology* 148:141-147.

Rodrigues, K.F. (1994). The foliar fungal endophytes of the Amazonian plam *Euterpe oleracea. Mycologia* 86:376-385.

Ruscoe, Q.W. (1971). Mycoflora of living and dead leaves of *Nothofagus truncata. Transactions of the British Mycological Society* 56:463-474.

Santamaria, J., Bayman, P. (2005). Fungal epiphytes and endophytes of coffee leaves *(Coffea arabica). Microbial Ecology* 50:1-8.

Saunders, M., Kohn, L.M. (2008). Host-synthesized compounds influence the in vitro interactions between fungal endophytes of maize. *Applied and Environmental Microbiology* 74:136-142.

Serrato-Diaz, L.M., Rivera-Vargas, L.I., Goenaga, R. (2010). First report of sooty mold of longan *(Dimocarpus longan* L.) caused by *Tripospermum porosporiferum*

Matsushima and *T. variabile* Matsushima in Puerto Rico. *Journal of Agriculture of the University of Puerto Rico* 94:285-287.

Sorensen, T. (1948). A method of establishing groups of equal amplitude in plant sociology based on similarity of species and its application to analyses of the vegetation on Danish commons. *Kongelige Danske Videnskabernes Selskab* 5:1-34.

Stone, J.K. (1987). Initiation and development of latent infetions by *Rhabdocline parkeri* on Douglas-fir. *Canadian Journal of Botany* 65:2614-2621.

Stone, J.K., Polishook, J.D., White, J.F. (2004). Endophytic fungi. In *Biodiversity of Fungi, Inventory and Monitoring Methods*, Mueller, G.M., Bills, G.F., Foster, M.S., Eds. Burlington, Elsevier Academic Press, pp 241-270.

Sun, X., Ding, Q., Hyde, K.D., Guo, L.D. (2012). Community structure and perference of endophytic fungi of three woody plants in a mixed forest. *Fungal Ecology* 5:624-632.

Tadych, M., Bergen, M.S., Johnson-Cicalese, J., Polashock, J.J., Vorsa, N., White, J.F. (2012). Endophytic and pathogenic fungi of developing cranberry ovaries from flower to mature fruit: diversity and succession. *Fungal Diversity* 54:101-116.

Taguchi, Y.H., Oono, Y. (2005). Relational patterns of gene expression via non-metric multidimensional scaling analysis. *Bioinformatics* 21:730-740.

Unterseher, M., Schnittler, M. (2009). Dilution-to-extinction cultivation of leaf-inhabiting endophytic fungi in beech (*Fagus sylvatica* L.) - different cultivation techniques influence fungal biodiversity assessment. *Mycological Research* 113:645-654.

Van Bael, S.A., Maynard, Z., Rojas, E., Mejia, L.C., Kyllo, D.A., Herre, E.A. (2005) Emerging perspectives on the ecological roles of endophytic fungi in tropical plants. In *The Fungal Community: Its Organization and Role in the Ecosystem*, Dighton, J., White, J.F., Oudemans, P., Eds. Boca Raton, Taylor and Francis group, pp. 181-192.

Vilgalys, R., Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172:4238-4246.

Watson E.S., McClurkin, D.C., Huneycutt, M.B. (1974). Fungal succession on loblolly pine and upland hardwood foliage and litter in North Mississippi. *Ecology* 55:1128-1134.

Wang, Y., Guo, L.D. (2007). A comparative study of endophytic fungi in needles, bark, and xylem of *Pinus tabulaeformis. Canadian Journal of Botany* 85:911-917.

Wearn, J.A., Sutton, B.C., Morley, N.J., Gange, A.C. (2012). Species and organ specificity of fungal endophytes in herbaceous grassland plants. *Journal of Ecology* 100:1085-1092.

Webster, J. (1956). Succession of fungi on decaying cocksfoot culms, Part I. *Journal of Ecology* 44:517-535.

Webster, J. (1957). Successoin of fungi on decaying cocksfoot culms, Part II. *Journal of Ecology* 45:1-30.

Wei, J.G., Xu, T., Guo, L.D., Liu, A.R., Zhang, Y., Pan, X.H. (2007). Endophytic *Pestalotiopsis* species associated with plants of Podocarpaceae, Theaceae, and Taxaceae in southern China. *Fungal Diversity* 24:55-74.

Whittaker, R.H. (1965) Dominance and diversity in land plant communities. *Science* 147:250-260.

White, T.J., Bruns, T., Lee, S., Taylor, J.W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: A Guide to Methods and Applications*, Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds. New York, Academic Press Inc., pp. 315-322.

Wildman, H.G., Parkinson, D. (1979). Microfungal succession on living leaves of *Populus tremuloides. Canadian Journal of Botany* 57:2800-2811.

Wrona, B., Grabowski, M. (2004). Etiology of apple sooty blotch in Poland. *Journal of Plant Protection Research* 44:293-297.

Yadav, A.S. (1966). The ecology of microfungi on decaying stems of *Heracleum sphondylium. Transactions of the British Mycological Society* 49:471-485.

Yang, X., Wilson, L.L., Madden, L.V., Ellis, M.A. (1990). Rain splash dispersal of *Colletotrichum acutatum* from infected strawberry fruit. *Phytopathology* 80:590-595.

Zimmerman, N.B., Vitousek, P.M. (2012). Fungal endophyte communities reflect environmental structuring across a Hawaiian landscape. *Proceedings of the National Academy of Sciences of the United States of America* 109:13022-13027.

Chapter 4

Avarez-Loayza, P., White, J.F., Torres, M.S., Balslev, H, Kristiansen, T., Svenning, J.C., (2011). Light converts endosymbiotic fungus to pathogen, influencing seedling survival and niche-space filling of a common tropical tree, *Iriartea deltoidea*. *PLoS ONE* 6:e16386.

Anderson, M.J., Walsh, D.C.I. (2013). PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing?. *Ecological Monographs* 83:557-574.

Andrews, J.H., Berbee, F.M., Nordheim, E.V. (1983). Microbial antagonism to the imperfect stage of apple scab pathogen, *Venturia inaequalis*. *Disease Control and Pest Management* 73:228-234.

Andrews, J.H., Harris, R.F. (2000). The ecology and biogeography of microorganisms on plant surfaces. *Annual Review of Phytopathology* 38:145-180.

Arnold, A.E., Mejia, L.C., Kyllo, D., Rojas, E.I., Maynard, Z., Robbins N., Herre, E.A. (2003). Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings*

of the National Academy of Sciences of the United States of America 100:15649-15654.

Breeze, E.M., Dix, N.J. (1981). Seasonal analysis of the fungal community on *Acer platanoides* leaves. *Transactions of the British Mycological Society* 77:321-328.

Brown, D.H., Di Meo, J.A. (1972). Influence of local maritime conditions on the distribution of two epiphytic lichens. *Lichenologist* 5:305-310.

Busby, P.E., Zimmerman, N., Weston, D.J., Jawdy, S.S., Houbraken, J., Newcombe, G. 2013. Leaf endophytes and Populus genotype affect severity of damage from the necrotropic leaf pathogen, *Drepanopeziza populi*. *Ecosphere,* 4:Art125.

Bussaban, B., Lumyong, S., Lumyong, P., McKenzie, E.H.C., Hyde, K.D. (2001). Endophytic fungi from *Amomum siamense*. *Canadian Journal of Microbiology* 47: 943-948.

Carroll, G., Petrini, O. (1983). Patterns of substrate utilization by some fungal endophytes from coniferous foliage. *Mycologia* 75:53-63.

Chapela, I.H., Boddy, L. (1988). Fungal colonization of attached beech branches I. Early stages of development of fungal communities. *New Phytologist* 110:39-45.

Chrysler, M.A., (1930). The origin and development of the vegetation of Sandy Hook. *Bulletin of the Torrey Botanical Club* 57:163-176.

Clarke, B.B., White Jr., J.F., Hurley, R.H., Torres, M.H., Sun, S., Huff, D.R. (2006). Endophyte-mediated suppression of dollar spot disease in fine fescues. *Plant Disease* 90:994-998.

Clarke, K.R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* 18:117-143.

Collado, J., Platas, G., Gonzalez, I., Palaez, F. (1999). Geographical and seasonal influences on the distribution of fungal endophytes in *Quercus ilex*. *New Phytologist* 144:525-532.

Collins, B.R., Anderson, K.H. (1994). *Plant Communities of New Jersey.* New Brunswick, Rutgers University Press, 287 pp.

Colwell, R.K. (2013). *EstimateS: Statistical estimation of species richness and shared species from samples. Version 9.* User's guide and application published at: http://purl.oclc.org/estimates.

Colwell, R.K., Mao, C.X., Chang, J. (2004). Interpolating, extrapolating, and comparing incidence-based species accumulation curves. *Ecology* 85:2717-2727.

Damm, U., Cannon, P.F., Woudenberg, J.H.C., Crous, P.W. (2012). The *Colletotrichum acutatum* species complex. *Studies in Mycology* 73:37-113.

Damm, U., Cannon, P.F., Woudenberg, J.H.C., Johnston, P.R., Weir, B.S., Tan, Y.P., Shivas, R.G., Crous, P.W. (2012). The *Colletotrichum boninense* species complex. *Studies in Mycology* 73:1-36.

De Mendiburu, F. (2009). *Una herramienta de analisis estadistico para la investigacion agricola.* Tesis. Universidad Nacional de Ingenieria *(UNI-PERU).*

Dickinson, C.H. (1965). The mycoflora associated with *Halimione portulacoides*: III. Fungi on green and moribund leaves.. *Transactions of the British Mycological Society* 48:603-610.

Dingle, J., Mcgee, P.A. (2003). Some endophytic fungi reduce the density of pustules of *Puccinia recondita* f. sp. *tritici* in wheat. *Mycological Research* 107:310-316.

Ding, S.-T., Sun, B.-N., Wu, J.-Y., Li, X.-C. (2011). Miocene *Smilax* leaves and associated epiphyllous fungi from Zhejiang, East China and their paleoecological implications. *Review of Paleobotany and Palynology* 165:209-223.

Fisher, P.J., Petrini, O., Sutton, B.C. (1993). A comparative study of fungal endophytes in leaves, xylem and bark of *Eucalyptus* in Australia and England. *Sydowia* 45:338-345.

Flessa, F., Persoh, D., Rambold, G. (2012). Annuality of Central European deciduous tree leaves delimits community development of epifoliar pigmented fungi. *Fungal Ecology* 5:554-561.

Fokkema, N.J. (1971). Influence of pollen on saprophytic and pathogenic fungi on rye leaves. In *Ecology of Leaf Surface Micro-Organisms,* Preece, T.F., Dickinson, C.H. Eds. New York, Academic Press Inc., pp. 277-282.

Fokkema, N.J. (1991). The phyllosphere as an ecologically neglected milieu: a plant pathologist's point of view. In *Microbial Ecology of Leaves,* Andrews, J.H., Hirano, S.S., Eds. New York, Springer-Verlag, pp. 3-18.

Gange, A.C., Dey, S., Currie, A.F., Sutton, B.C. (2007). Site- and species-specific differences in endophyte occurrence in two herbaceous plants. *Journal of Ecology* 95:614-622.

Ganley, R.J., Sniezko, R.A., Newcombe, G. (2008). Endophyte mediated resistance against white pine blister rust in *Pinus monticola*. *Forest Ecology and Management* 255:2751-2760.

Gore, M.E., Bucak, C. (2007). Geographical and seasonal influences on the distribution of fungal endophytes *in Laurus nobilis*. *Forest Pathology* 37:281-288.

Guo, L.D., Huang, G.R., Wang, Y. (2008). Seasonal and tissue age influences on endophytic fungi of *Pinus tabulaeformis* (Pinaceae) in Donglong Mountains, Beijing. *Journal of Integrative Plant Biology* 50:997-1003.

Hammer, O., Harper, D.A.T., Ryan, P. D. (2001). PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica,* 4(1):1-9.

Harshberger, J.W. (1916). *The Vegetation of the New Jersey Pine Barrens.* New York, Dover Publications, pp. 329.

Hata, K., Futai, K. (1995). Endophytic fungi associated with healthy pine needles and needles infested by the pine needle gall midge, *Thecodiplosis japonensis*. *Canadian Journal of Botany* 73:384-390.

Hata, K., Futai, K., Tsuda, M. (1998). Seasonal and needle age-dependent changes of the endophytic mycobiota in *Pinus thunbergii* and *Pinus densiflora* needles. *Canadian Journal of Botany* 76:245-250.

He, X., Han, G., Lin, Y., Tian, X., Xiang, C., Tian, Q., Wang, F., He, Z. (2012). Diversity and decomposition potential of endophytes in leaves of *Cinnamomum camphora* plantation in China. *Ecological Research* 27:273-284.

Higgins, K.L., Arnold, A.E., Miadlikowska, J., Sarvate, S.D., Lutzoni, F. (2007). Phylogenetic relationships, host affinity, and geographic structure of boreal and arctic endophytes from three major plant lineages. *Molecular Phylogenetics* 42: 543-555.

Hutchinson, G.E. (1957). Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology* 22:415-427.

Istifadah, N., McGee, P.A. (2006). Endophytic *Chaetomium globosum* reduces development of tan spot in wheat caused by *Pyrenophora tritici-repentis*. *Australasian Plant Pathology* 35:411-418.

Jumpponen, A., Jones, K.L. (2010). Seasonally dynamic fungal communities in the *Quercus macrocarpa* phyllosphere differ between urban and nonurban environments. *New Phytologistogist* 186:496-513.

Kembel, S.W., Mueller, R.C. (2014). Plant traits and taxonomy drive host associations in tropical phyllosphere fungal communities. *Botany* 92:303-311.

Koide, K., Osono, T., Takeda, H. (2005). Colonization and lignin decomposition of *Cammellia japonica* leaf ltter by endophytic fungi. *Mycoscience* 46:280-286.

Kowalski, K.P., Bacon, C., Bickford, W., Braun, H. Clay, K., Leduc-Lapierre, M., Lillard, E., McCormick, M., Nelson, E., Torres, M., White, J., Wilcox, D.A. (2015). Advancing the science of microbial symbiosis to support invasive species management: A case study on *Phragmites* in the Great Lakes. *Frontiers in Microbiology* - Volume in press. (2015 Feb 19; 6:95. doi: 10.3389/fmicb.2015.00095. eCollection 2015).

Kuldau, G., Bacon, C. (2008). Clavicipitaceous endophytes: Their ability to enhance resistance of grasses to multiple stresses. *Biological Control* 46:57-71.

Kumar, D.S.S., Hyde, K.D. (2004). Biodiversity and tissue-recurrence of endophytic fungi in *Tripterygium wilfordii*. *Fungal Diversity* 17:69-90.

Kurose, D., Furuya, N., Tsuchiya, K., Tsushima, S., Evans, H.C. (2012). Endophytic fungi associated with *Fallopia japonica* (Polygonaceae) in Japan and their interactions with *Puccinia polygoni-amphibii* var. *tovariae,* a candidate for classical biological control. *Fungal Biology* 116:785-791.

Lee, K., Pan, J.J., May, G. (2009). Endophytic *Fusarium verticilloides* reduces disease severity caused by Ustilago maydis on maize. *FEMS Microbiology Letters* 299:31-37.

Legault, D., Dessureault, M., Laflamme, G. (1989). Mycoflora of *Pinus banksiana* and *Pinus resinosa* needles. II. Epiphytic fungi. *Canadian Journal of Botany* 67:2061-2065.

Ludlum, D.M. (1983). *The New Jersey Weather Book.* New Brunswick, Rutgers University Press, pp. 249.

Luttrell, E.S. (1944). The morphology of *Myiocopron smilacis* (De. Not.) Sacc. *American Journal of Botany* 31:640-649.

Magurran, A.E. (2004). *Measuring Biological Diversity.* Malden, Blackwell Publishing company, 256 pp.

Mishra, A., Gond, S.K., Kumar, A., Sharma, V.K., Verma, S.K., Kharwar, R.N., Sieber, T.N. (2012). Season and tissue type affect fungal endophyte communities of the Indian medicinal plant *Tinospora cordifolia* more strongly than geographic location. *Microbial Ecology* 64:388-398.

Mishra, R.R., Dickinson, C.H. (1981). Phylloplane and litter fungi of *Ilex aquifolium*. *Transactions of the British Mycological Society* 77:329-337.

Morin, L., Auld, B.A., Brown, J.F. (1993a). Interaction between *Puccinia xanthii* and facultative parasitic fungi on *Xanthium occidentale*. *Biological Control* 3:288-295.

Morin, L., Auld, B.A., Brown, J.F. (1993b). Synergy between *Puccinia xanthii* and *Colletotrichum orbiculare* on *Xanthium occidentale*. *Biological Control* 3:296-310.

Muvea, A.M., Meyhofer, R., Subramanian, S., Poehling, H.-M., Ekesi, S., Maniania, N.K. (2014). Colonization of onions by endophytic fungi and their impacts on the biology of *Thrips tabaci. PLoS ONE* 9:e108242.

Osono T., Hirose, D. (2009). Effects of prior decomposition of *Camellia japonica* leaf litter by an endophytic fungus on the subsequent decomposition by fungal colonizers. *Mycoscience* 50:52-55.

Osono, T. (2002). Phyllosphere fungi on leaf litter of *Fagus crenata*: occurrence, colonization, and succession. *Canadian Journal of Botany* 80:460-469.

Osono, T. (2006). Role of phyllosphere fungi of forest trees in the development of decomposer fungal communities and decomposition processes of leaf litter. *Canadian Journal of Botany* 52:701-716.

Osono, T. (2008). Endophytic and epiphytic phyllosphere fungi of *Camellia japonica*: seasonal and leaf age-dependent variations. *Mycologia* 100:387-391.

Osono, T. (2014). Diversity and ecology of endophytic and epiphytic fungi of tree leaves in Japan: a review. In *Advances in Endophytic Research,* Verma, V.C., Gange, A.C., Eds. Springer Science and Business Media, pp. 1-26.

Osono, T., Mori, A. (2004). Distribution of fungi within the canopy of giant dogwood. *Mycoscience* 45:161-168.

Pereira, P.T., DeCarvalho, M.M., Girio, F.M., Roseiro, J.C., Amaral-Collaco, M.T. (2002). Diversity of microfungi in the phylloplane of plants growing in a Mediterranean ecosystem. *Journal of Basic Microbiology* 42:396-407.

Perello, A., Simon, M.R., Arambarri, A.M. (2002). Interactions between foliar pathogens and saprophytic microflora of the wheat (*Triticum aestivum* L.) phylloplane. *Journal of Phytopathology* 150:232-243.

Persoh, D. (2013). Factors shaping community structure of endophytic fungi-evidence from the *Pinus*-*Viscum*-system. *Fungal Diversity* 60:55-69.

Petrini, O. (1996). Ecological and Physiological Aspects of Host-Specificity in Endophytic Fungi. In *Endophytic Fungi in Grasses and Woody Plants,* Redlin, S.C., Carris, L.M., Eds., St. Paul, APS Press, pp. 87-100.

Photita, W., Lumyong, S., Lumyong, P., Hyde, K.D. (2001). Endophytic fungi of wild banana (*Musa acuminata*) at Doi Suthep Pui National Park, Thailand. *Mycological Research* 105:1508-1513.

Photita, W., Lumyong, S., Lumyong, P., McKenzie, E. H., & Hyde, K. D. (2004). Are some endophytes of *Musa acuminata* latent pathogens. *Fungal Diversity* 16:131-140.

Promputtha, I., Hyde, K. D., McKenzie, E. H., Peberdy, J. F., & Lumyong, S. (2010). Can leaf degrading enzymes provide evidence that endophytic fungi becoming saprobes? *Fungal Diversity* 41:89-99.

Pugh, G.J.F., Buckley, N.G. (1971). The leaf surface as a substrate for colonization by fungi. In *Ecology of Leaf Surface Micro-organisms,* Preece, T.F., Dickinson, C.H., New York, Academic Press Inc., pp. 431-445.

Purahong, W., Hyde, K. (2011). Effects of fungal endophytes on grass and non-grass litter decomposition rates. *Fungal Diversity* 47:1-7.

Raghavendra, A.K.H., Newcombe, G. (2013). The contribution of foliar endophytes to quantitative resistance to *Melampsora* rust. *New Phytologist* 197:909-918.

Ridout, M., Newcombe, G. (2015). The frequency of modification of *Dothistroma* pine needle blight severity by fungi within the native range. *Forest Ecology and Management* 337:153-160.

Rollinger, J.L., Langenheim, J.H. (1993). Geographic survey of fungal endophyte community composition in leaves of Coastal Redwood. *Mycologia* 85:149-156.

Santamaria, O., Diez, J J. (2005). Fungi in leaves, twigs, and stem bark of *Populus tremula* from northern Spain. *Forest Pathology* 35:95-104.

Santamaria, J., Bayman, P. (2005). Fungal epiphytes and endophytes of coffee leaves (*Coffea arabica*). *Microbial Ecology* 50:1-8.

Saunders, M., Kohn, L.M. (2009). Evidence for alteration of fungal endophyte community assembly by host defense compounds. *New Phytologist* 182:229-238.

Sun, X., Ding, Q., Hyde, K.D., Guo, L.D. (2012). Community structure and preference of endophytic fungi of three woody plants in a mixed forest. *Fungal Ecology* 5: 624-632.

Sun, X., Guo, L.-D., Hyde, K.D. (2011). Community composition of endophytic fungi in *Acer truncatum* and their role in decomposition. *Fungal Diversity* 47:85-95.

Suryanarayanan, T.S., Senthilarasu, G., Muruganandam, V. (2000). Endophytic fungi from *Cuscuta reflexa* and its host plants. *Fungal Diversity* 4:117-123.

Tadych, M., Bergen, M. S., Johnson-Cicalese, J., Polashock, J. J., Vorsa, N., White, J. F. (2012). Endophytic and pathogenic fungi of developing cranberry ovaries from flower to mature fruit: diversity and succession. *Fungal Diversity* 54:101-116.

Tukey, Jr., H.B., (1971). Leaching of substances from Plants. In *Ecology of Leaf Surface Micro-Organisms*, Preece, T.F., Dickinson, C.H., Eds., New York: Academic Press Inc., pp. 67-80.

Unterseher, M. (2011). Diversity of fungal endophytes in temperate forest trees. In *Endophytes of Forest Trees: Biology and Applications,* Pirttila, A.M., Frank, A.C., Eds., accessed online (doi:10.1007/978-94-007-1599-8_2.), Springer Netherlands, pp. 31-46.

Unterseher, M., Reiher, A., Finstermeier, K., Otto, P., Morawetz, W. (2007). Species richness and distribution patterns of leaf-inhabiting endophytic fungi in a temperate forest canopy. *Mycological Progress* 6:201-212.

Verma, V. C., Gond, S.K., Kumar, A., Kharwar, R.N., Strobel, G. (2007). The endophytic mycoflora of bark, leaf, and stem tissues of *Azadirachta indica* A. Juss (Neem) from Varanasi (India). *Microbial Ecology* 54:119-125.

Wang, Y., Guo, L.D. (2007). A comparative study of endophytic fungi in needles, bark, and xylem of *Pinus tabulaeformis*. *Canadian Journal of Botany* 85:171-184.

Zambell, C.B., White, J.F. (2014). In the forest vine *Smilax rotundifolia*, fungal epiphytes show site-wide spatial correlation, while endophytes show evidence of niche partitioning. *Fungal Diversity*. doi:10.1007/s13225-014-0316-3

Zamora, P., Martinez-Ruiz, C., Diez, J.J. (2008). Fungi in needles and twigs of pine plantations from northern Spain. *Fungal Diversity* 30:171-184.

Chapter 5

Adhikari, K.B., Tanwir, F., Gregersen, P.L., Steffensen, S.K., Jensen, B.M., Poulsen, L.K., Nielsen, C.H., Hoyer, S., Borre, M., Fomsgaard, I.S. (2015). Benzoxazinoids: Cereal phytochemicals with putative therapeutic and health-protecting properties. *Molecular Nutrition & Food Research* 0:1-5

Ahmed, M.A. (1986). Behavior of phyllosphere fungi on maize leaves in Egypt. *New Letter Microbiological Resource Centre, Cairo, Egypt* 11:9-16.

Andrews, J.H., Berbee, F.M., Nordheim, E.V. (1983). Microbial antagonism to the imperfect stage of apple scab pathogen, *Venturia inaequalis*. *Disease Control and Pest Management* 73:228-234.

Arnold, A.E. (2007). Understanding the diversity of foliar endophytic fungi: progress, challenges, frontiers. *Fungal Biology Reviews* 21:51-66.

Arnold, A.E., Herre, E.A. (2003). Canopy cover and leaf age affect colonization by tropical fungal endophytes: ecological pattern and process in *Theobroma cacao* (Malvaceae). *Mycologia* 95:388-398.

Arnold, A.E., Lutzoni, F. (2007). Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? *Ecology* 88:541-549.

Arnold, A.E., Maynard, Z., Gilbert, G.S. (2001). Fungal endophytes in dicotyledonous neotropical trees: patterns of abundance and diversity. *Mycological Research* 105:1502-1507.\

Arnold, A.E., Mejia, L.C., Kyllo, D., Rojas, E.I., Maynard, Z., Robbins, N., Herre, E.A. (2003). Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences of the United States of America* 100:15649-15654.

Bailey, J. K., Deckert, R., Schweitzer, J.A., Rehill, B.J., Lindroth, R.L., Gehring, C., Whitham, T.G. (2005). Host plant genetics affect hidden ecological players: links among *Populus*, condensed tannins, and fungal endophyte infection. *Canadian Journal of Botany* 83:356-361.

Balint, M., Tiffin, P., Hallstrom, B., O'Hara, R.B., Olson, M.S., Frankhauser, J.D., Piepenbring, M., Schmitt, I. (2013). Host genotype shapes the foliar fungal microbime of balsam poplar *(Populus balsamifera). PLoS One* 8:e53987.

Bernstein, M.E., Carroll, G.C. (1977). Internal fungi in old-growth Douglas fir foliage. *Canadian Journal of Botany* 55:644-653.

Boyle, C., Gotz, M., Dammann-Tugend, U., Schulz, B. (2001). Endophyte-host interactions III. local vs. systemic colonization. *Symbiosis* 31:259-281.

Busby, P.E., Zimmerman, N., Weston, D.J., Jawdy, S.S., Houbraken, J., Newcombe, G. (2013). Leaf endophytes and *Populus* genotype affect severity of damage from the necrotropic leaf pathogen, *Drepanopeziza populi*. *Ecosphere* 4: Art125. doi:http://dx.doi.org/10.1890/ES13-00127.1

Cabral, D. (1985). Phyllosphere of *Eucalyptus viminalis*: dynamics of fungal populations. *Transactions of the British Mycological Society* 85:501-511.

Cabral, D., Stone, J., Carroll, G.C. (1993). The internal mycoflora of *Juncus* spp.: microscopic and cultural observation of infection patterns. *Mycological Research* 97:367-376.

Caretta, G., Frate, G., Franca, P., Guglielminetti, M., Mangiarotti, A.M., Savino, E. (1985). Fora fungina del mais: funghi del terreno, del filloplano e spore dell'aria. *Archivo Botanica e Biogeografico Italiano* 61:143-168.

Carroll, F.E., Muller, E., Sutton, B.C. (1977). Preliminary studies on the incidence of needle endophytes in some European conifers. *Sydowia* 29:87-103.

Carroll, G. (1988). Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. *Ecology* 69:2-9.

Carroll, G.C., Carroll, F.E. (1978). Studies on the incidence of coniferous needle endophytes in the Pacific Northwest. *Canadian Journal of Botany* 56:3034-3043.

Carroll, G., Petrini, O. (1983). Patterns of substrate utilization by some fungal endophytes from coniferous foliage. *Mycologia* 75:53-63.

Cavender-Bares, J., Ackerly, D.D., Baum, D.A., Bazzaz, F.A. (2004). Phylogenetic overdispersion in Floridian Oak communities. *The American Naturalist* 163: 823-843.

Cavender-Bares, J., Keen, A., Miles, B. (2006). Phylogenetic structure of Floridian plant communities depends on taxonomic and spatial scale. *Ecology* 87: S109-S122.

Chareprasert, S., Piapukiew, J., Thienhirun, S., Whalley, A.J., Sihanonth, P. (2006). Endophytic fungi of teak leaves *Tectona grandis* L. and rain tree leaves *Samanea saman* Merr. *World Journal of Microbiology & Biotechnology* 22:481-486.

Clarke, B.B., White Jr. , J.F., Hurley, R.H., Torres, M.H., Sun, S., & Huff, D.R. (2006). Endophyte-mediated suppression of dollar spot disease in fine fescues. *Plant Disease* 90:994-998.

Coley, P.D., Barone, J.A. (1996). Herbivory and plant defensis in tropical forests. *Annual Review of Ecology and Systematics* 27:305-335.

Connell, J.H. (1961). The influence of interspecific competition and other factors on the distribution of the barnacle *Chamalus stellatus*. *Ecology*. 42:710-723.

Dingle, J., Mcgee, P.A. (2003). Some endophytic fungi reduce the density of pustules of *Puccinia recondita* f. sp. *tritici* in wheat. *Mycological Research* 107: 310-316.

Douanla-Meli, C., Langer, E., Talontsi Mouafo, F. (2013). Fungal endophyte diversity and community patterns in healthy and yellowing leaves of *Citrus limon*. *Fungal Ecology* 6: 212-222.

Elton, C.S. (1946). Competition and structure of ecological communities. Journal of *Animal Ecology* 15: 54-68.

Espinosa-Garcia, F.J., Langenheim, J.H. (1991). Effect of some leaf essential oil phenotypes from coastal redwood on the growth of its predominant endophytic fungus, *Pleuroplaconema* sp. *Journal of Chemical Ecology* 19:629-642.

Espinosa-Garcia, F.J., Rollinger, J.L., Langenheim, J.H. (1996). Coastal redwood leaf endophytes: their occurrence, interactions and response to host vaolatile terpenoids. In *Endophytic fungi in grasses and woody plants,* Redlin, S.C., Carris, L.M., Eds. St. Paul, The American Phytopathological Society, pp. 101-120.

Estrada, C., Wcislo, W.T., Van Bael, S.A. (2013). Symbiotic fungi alter plant chemistry that discourages leaf-cutting ants. *New Phytologist* 198:241-251.

Ganley, R..J., Brunsfeld, S.J., Newcombe, G. (2004). A community of unknown, endophytic fungi in western white pine. *Proceedings of the National Academy of Sciences of the United States of America* 101:10107-10112.

Ganley, R.J., Sniezko, R.A., Newcombe, G. (2008). Endophyte mediated resistance against white pine blister rust in *Pinus monticola*. *Forest Ecology and Management* 255:2751-2760.

Gazis, R., Rehner, S., Chaverri, P. (2011). Species delmitation in fungal endophyte diversity studies and its implications in ecological and biogeographic inferences. *Molecular Ecology* 20:3001-3013.

Glienke, C., Pereira, O. L., Stringari, D., Fabris, J., Kava-Cordeiro, V., Galli-Terasawa, L., Cunnington, J., Shiva, R.G., Groenewald, J.Z., Crous, P.W (2011). Endophytic and pathogenic *Phyllosticta* species, with reference to those associated with Citrus Black Spot. *Persoonia* 26:47-56.

Gonzalez, V., Tello, M.L. (2011). The endophytic mycota associated with *Vitis vinifera*. *Fungal Diversityity* 47:29-42.

Gravel, D., Canham, C.D., Beaudet, M., Messier, C. (2006). Reconciling niche and neutrality: the continuum hypothesis. *Ecology Letters* 9:399-409.

Hammond, K.E., Lewis, B.G., Musa, T.M. (1985). A systemic pathway in the infection of oilseed rape plants by *Leptosphaeria maculans*. *Plant Pathology* 34: 557-565.

Hata, K., Futai, K. (1995). Endophytic fungi associated with healthy pine needles and needles infested by the pine needle gall midge, *Thecodiplosis japonensis*. *Canadian Journal of Botany* 73:384-390.

Hata, K., Futai, K. (1996). Variation in fungal endophyte populations in needles of the genus *Pinus*. *Canadian Journal of Botany* 74:103-114.

Hata, K., Futai, K., Tsuda, M. (1998). Seasonal and needle age-dependent changes of the endophytic mycobiota in Pinus thunbergii and *Pinus densiflora* needles. *Canadian Journal of Botany* 76:245-250.

He, X., Han, G., Lin, Y., Tian, X., Xiang, C., Tian, Q., Wang, F., He, Z. (2012). Diversity and decomposition potential of endophytes in leaves of *Cinnamomum camphora* plantation in China. *Ecological Research* 27:273-284.

Hodgson, S., Cates, C., Hodgson, J., Morley, N.J., Sutton, B.C., Gange, A.C. (2014). Vertical transmission of fungal endophytes is widespread in forbs. *Ecology and Evolution* 4:1199-1208.

Hoffland, E., Niemann, G.J., Van Pelt, J.A., Prueveen, J.B., Eijkel, G.B., Boon, J.J., Lambers, H. (1996). Relative growth rate correlates negatively with pathogen resistance in radish: the role of plant chemistry. *Plant, Cell and Environment* 19: 1281-1290.

Hubbell, S.P. (1979). Tree dispersion, abundance and diversity in a tropical dry forest. *Science* 203:1299-1309.

Hussain, A.I., Anwar, F., Sherazi, S.T., Przybylski, R. (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry* 108:986-995.

Hutchinson, G.E. (1978). *An Introduction to Population Ecology*. New Haven, Yale University Press, 260 pp.

Hyde, K.D., Soytong, K. (2008). The fungal endophyte dilemma. *Fungal Diversity* 33:163-173.

Istifadah, N., McGee, P.A. (2006). Endophytic *Chaetomium globosum* reduces development of tan spot in wheat caused by *Pyrenophora tritici-repentis*. *Australasian Plant Pathology* 35:411-418.

Juniper, B.E. (1991). The Leaf from the Inside and Outside: A Microbe's Perspective. In *Microbial Ecology of Leaves,* Andrews, J.H., Hirano, S.S., Eds., New York, Springer-Verlag, pp. 21-42.

Kembel, S.W., Mueller, R.C. (2014). Plant traits and taxonomy drive host associations in tropical phyllosphere fungal communities. *Botany* 92:303-311.

Kendrick, B., Burges, A. (1962). Biological aspects of decay of *Pinus sylvestris* leaf litter. *Nova Hedwigia* 4:313-342.

Koide, K., Osono, T., Takeda, H. (2005). Colonization and lignin decomposition of *Cammellia japonica* leaf ltter by endophytic fungi. *Mycoscience* 46:280-286.

Krings, M., Taylor, T.N., Dotzler, N. (2012). Fungal endophytes as a driving force in land plant evolution: evidence from the fossil record. In *Biocomplexity of Plant-Fungal Interactions,* D. Southworth, Ed., Wiley Online Library (DOI: 10.1002/9781118314364.ch1), John Wiley & Sons Inc., pp. 5-28.

Kuldau, G., Bacon, C. (2008). Clavicipitaceous endophytes: Their ability to enhance resistance of grasses to multiple stresses. *Biological Control* 46:57-71.

Kumar, D.S., Hyde, K.D. (2004). Biodiversity and tissue-recurrence of endophytic fungi in *Tripterygium wilfordii. Fungal Diversity* 17:69-90.

Kurose, D., Furuya, N., Tsuchiya, K., Tsushima , S., Evans, H.C. (2012). Endophytic fungi associated with *Fallopia japonica* (Polygonaceae) in Japan and their interactions with *Puccinia polygoni-amphibii* var. *tovariae,* a candidate for classical biological control. *Fungal Biology* 116:785-791.

Lambers, H., Chapin III, F.S., Pons, T.L. (2008). *Plant Physiological Ecology*, 2nd ed., New York, Springer, 604 pp.

Last, F.T., Warren, R.C. (1972). Non-parasitic microbes colonizing green leaves: their form and functions. *Endeavour* 31:143-150.

Laube, S., Zotz, G. (2006). Neither host-specific nor random: vascular epiphytes on three tree species in a Panamanian lowland forest. *Annals of Botany* 97:1103-1114.

Lee, K., Pan, J.J., May, G. (2009). Endophytic *Fusarium verticilloides* reduces disease severity caused by *Ustilago maydis* on maize. *FEMS Microbiology Letters* 299:31-37.

Lindow, S.E., Brandl, M.T. (2003). Microbiology of the Phyllosphere. *Applied and Environmental Microbiology* 69:1875-1883.

Macauley, B.J., Thrower, L.B. (1966). Succession of fungi in leaf litter of *Eucalyptus regnans*. *Transactions of the British Mycological Society* 49:509-520.

Magurran, A.E. (2004). *Measuring Biological Diversity.* Malden, Blackwell Publishing company, 256 pp.

McGee, P.A. (2002). Reduced growth and deterrence from feeding of hte insect pest *Helicoverpa armigera* associated with fungal endophytes from cotton. *Animal Production Science* 42:995-999.

McGill, B.J., Etienne, R.S., Gray, J.S., Alonso, D., Anderson , M.J., Benecha, H.K., Habtamu, K., Dornelas, M., Enquist, B. J., Green, J.L., He, F., Hurlbert, A.H., Magurran, A.E., Marquet, P.A., Maurer, B.A., Ostling, A., Soykan, C.U., Ugland, K.I., White, E. P. (2007). Species abundance distributions: moving beyond single prediction theories to integration within an ecological framework. *Ecology Letters* 10:995-1015.

Meredith, D.S. (1963). *Pyricularia grisea* (Cooke) Sacc. causing pitting disease of bananas in Central America. *Annals of Applied Biology* 52:453-463.

Mishra, R.R., Dickinson, C.H. (1981). Phylloplane and litter fungi of *Ilex aquifolium*. *Transactions of the British Mycological Society* 77:329-337.

Mohandoss, J., Suryanarayanan, T.S. (2009). Effect of fungicide treatment on foliar fungal endophyte diversity in mango. *Sydowia* 61:11-24.

Morin, L., Auld, B.A., Brown, J.F. (1993). Interaction between Puccinia xanthii and facultative parasitic fungi on Xanthium occidentale. *Biological Control* 3:288-295.

Morin, L., Auld, B.A., Brown, J.F. (1993). Synergy between *Puccinia xanthii* and *Colletotrichum orbiculare* on *Xanthium occidentale*. *Biological Control* 3:296-310.

Mostert, L., Crous, P.W., Petrini, O. (2000). Endophytic fungi associated with shoots and leaves of Vitis vinifera, with specific reference to the *Phomopsis viticola* complex. *Sydowia* 52:46-58.

Mucciarelli , M., Scannerini, S., Bertea, C., Maffei, M. (2003). In vitro and in vivo peppermint growth promotion by nonmycorrhizal fungal colonization. *New Phytologist* 158:579-591.

Mucciarelli, M., Camusso, W., Maffei, M., Panicco, P., Bicchi, C. (2007). Volatile terpenoids of endophyt-free and infected peppermint (*Mentha piperita* L.): chemical partitioning of a symbiosis. *Microbial Ecology* 54:685-696.

Mucciarelli, M., Scannerini, S., Bertea, C.M., Maffei, M. (2002). An ascomycetous endophyte isolated from Mentha piperita L.: biological features and molecular studies. *Mycologia* 94:28-39.

Munoz, A.A., Chacon, P., Perez, F., Barnert, E.S., Armesto, J.J. (2003). Diverstiy and host tree preferences of vascular epiphytes and vines in a temperate rainforest in sourthern Chile. *Australian Journal of Botany* 51:381-391.

Muvea, A.M., Meyhofer, R., Subramanian, S., Poehling, H.-M., Ekesi, S., Maniania, N. K. (2014). Colonization of onions by endophytic fungi and their impacts on the biology of *Thrips tabaci*. *PLoS One* 9:e108242.

Okane, I., Srikitikulchai, P., Tabuchi, Y., Sivichai, S., Nakagiri, A. (2012). Recognition and characterization of four Thai xylariaceous fungi inhabiting various tropical foliages as endophytes by DNA sequences and host plant preference. *Mycoscience* 53:122-132.

Osbourn, A.E. (1996). Preformed antimicrobial compounds and plant defense against fungal attack. *The Plant Cell* 8:1821-1831.

Osono, T., Bhatta, B.K., Takeda, H. (2004). Phyllosphere fungi on living and decomposing leaves of giant dogwood. *Mycoscience* 45:35-41.

Osono, T. (2002). Phyllosphere fungi on leaf litter of *Fagus crenata*: occurrence, colonization, and succession. *Canadian Journal of Botany* 80:460-469.

Osono, T. (2006). Role of phyllosphere fungi of forest trees in the development of decomposer fungal communities and decomposition processes of leaf litter. *Canadian Journal of Botany* 52:701-716.

Osono, T. (2008). Endophytic and epiphytic phyllosphere fungi of *Camellia japonica*: seasonal and leaf age-dependent variations. *Mycologia* 100:387-391.

Osono, T., Mori, A. (2004). Distribution of fungi within the canopy of giant dogwood. *Mycoscience* 45:161-168.

Perello, A., Simon, M.R., Arambarri, A.M. (2002). Interactions between foliar pathogens and saprophytic microflora of the wheat (*Triticum aestivum* L.) phylloplane. *Journal of Phytopathology* 150:232-243.

Persoh, D. (2013). Factors shaping community structure of endophytic fungi-evidence from the *Pinus*-*Viscum*-system. *Fungal Diversity* 60:55-69.

Petrini, O. (1991). Fungal endophytes of tree leaves. In *Microbial Ecology of Leaves,* Andrews, J.H., Hirano, S.S., Eds. New York, Springer, pp. 179-197.

Petrini, O. (1996). Ecological and physiological aspects of host-specificity in endophytic fungi. In *Endophytic Fungi in Grasses and Woody Plants.* Redlin, S.C., Carris, L.M., Eds. St. Paul, APS Press, pp. 87-100.

Petrini, O., Stone, J., Carroll, F.E. (1982). Endophytic fungi in evergreen shrubs in western Oregon: A preliminary study. *Canadian Journal of Botany* 60:789-796.

Photita, W., Lumyong, S., Lumyong, P., Hyde, K.D. (2001). Endophytic fungi of wild banana (*Musa acuminata*) at Doi Suthep Pui National Park, Thailand. *Mycological Research* 105:1508-1513.

Photita, W., Lumyong, S., Lumyong, P., McKenzie, E.H., Hyde, K. D. (2004). Are some endophytes of *Musa acuminata* latent pathogens. *Fungal Diversityity* 16:131-140.

Piasecka , A., Jedrzejczak-Rey, N., Bednarek, P. (2015). Secondary metabolites in plant innate immunity: conserved function of divergent chemicals. *New Phytologist* 206:948-964.

Porras-Alfaro, A., Bayman, P. (2011). Hidden fungi, emergent properties: endophytes and microbiomes. *Annual Review of Phytopathology* 49:291-315.

Promputtha, I., Hyde, K. D., McKenzie, E. H., Peberdy, J. F., Lumyong, S. (2010). Can leaf degrading enzymes provide evidence that endophytic fungi becoming saprobes? *Fungal Diversity* 41:89-99.

Promputtha, I., Lumyong, S., Vijaykrishna, D., McKenzie, E.H., Hyde, K., Jeewon, R. (2007). A phylogenetic evaluation of whether endophytes become saprotrophs at host senescence. *Microbial Ecology* 53:579-590.

Prusky, D. (1996). Pathogen quiescence in postharvest diseases. *Annual Review of Phytopathology* 34:413-434.

Prusky, D., Alkan, N., Mengiste, T., Fluhr, R. (2013). Quiescent and necrotrophic lifestyle choice during postharvest disease development. *Annual Review of Phytopathology* 51:155-176.

Pscheidt, J.W., Pearson, R.C. (1989). Time of infection and control of *Phomopsis* fruit rot of grape. *Plant Disease* 73:829-833.

Raghavendra, A.K., Newcombe, G. (2013). The contribution of foliar endophytes to quantitative resistance to *Melampsora* rust. *New Phytologist* 197:909-918.

Ridout, M., Newcombe, G. (2015). The frequency of modification of *Dothistroma* pine needle blight severity by fungi within the native range. *Forest Ecology and Management* 337:153-160.

Rodriguez, R.J., White Jr., J.F., Arnold, A.E., Redman, R.S. (2009). Fungal endophytes: diversity and functional roles. *New Phytologist* 182:314-330.

Rohloff, J. (1999). Monoterpene composition of essential oil from peppermint (*Mentha* x *piperita* L.) with regard to leaf position using solid-phase microextraction and gas chromatography/mass spectrophotometry analysis. *Journal of Agricultural and Food Chemistry* 47:3782-3786.

Rojas, E. I., Rehner, S. A., Samuels, G. J., Van Bael, S. A., Herre, E. A., Cannon, P., Chen, R., Pang, J., Wang, R., Zhang, Y., Peng, Y.-Q., Sha, T. (2010). *Colletotrichum gloeosporioides* s.l. associated with *Theobroma cacao* and other plants in Panama: multilocus phylogenies distinguish host-associated pathogens from asymptomatic endophytes. *Mycologia* 102:1328-1338.

Ruscoe, Q.W. (1971). Mycoflora of living and dead leaves of *Nothofagus truncata*. *Transactions of the British Mycological Society* 56:463-474.

Saikkonen, K. (2007). Forest structure and fungal endophytes. *Fungal Biology* 21:67-74.

Sanchez-Azofeifa, A., Oki, Y., Fernandes, G.W., Ball, R.A., Gamon, J. (2012). Relationhips between endophyte diversity and leaf optical properties. *Trees* 26:291-299.

Santamaria, J., Bayman, P. (2005). Fungal epiphytes and endophytes of coffee leaves (*Coffea arabica*). *Microbial Ecology* 50:1-8.

Saunders, M., Kohn, L.M. (2009). Evidence for alteration of fungal endophyte community assembly by host defense compounds. *New Phytologist* 182:229-238.

Saunders, M., Glenn, A.E., Kohn, L.M. (2010). Exploring the evolutionary ecology of fungal endophytes in agricultural systems: using functional traits to reveal mechanisms in community processes. *Evolutionary Applications* 3:525-537.

Schulz, B., Boyle, C. (2005). The endophytic continuum. *Mycological Research* 109:661-686.

Schulz, B., Boyle, C. (2006). What are endophytes? In *Microbial Root Endophytes (Soil Biology, Volume 9),* Schulz, B.C., Boyle, C.J., Sieber, T.N., Eds., accessed online (doi:10.1007/3-540-33526-9), Springer-Verlag, pp. 1-13.

Sherwood-Pike, M., Stone, J.K., Carroll, G.C. (1986). *Rhabdocline parkeri*, a ubiquitous foliar endophyte of Douglas-fir. *Canadian Journal of Botany* 64:1849-1855.

Shubin, L., Juan, H., Renchao, Z., ShiRu, X., YuanXiao, J. (2014). Fungal endophytes of *Alpinia officinarum* rhizomes: insights on diversity and variation across growth years, growth sites, and the inner active chemical concentration. *PLoS ONE* 9:e115289.

Sieber, T.N. (2007). Endophytic fungi in forest trees: are they mutualists? *Fungal Biology Reviews* 21:75-89.

Simberloff, D.S. (1970). Taxonomic diversity of island biotas. *Evolution* 24: 23-47.

Sinclair, J.B., Cerkauskas, R.F. (1996). Latent infection vs. endophytic colonization by fungi. In *Endophytic fungi in grasses and woody plants,* Redlin, S.C., Carris, L.M. Eds., St. Paul, APS Press, pp. 3-29.

Slippers, B., Wingfield, M.J. (2007). Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews* 21:90-106.

Stone, J.K. (1987). Initiation and development of latent infections *by Rhabdocline parkeri* on Douglas-fir. *Canadian Journal of Botany* 65:2614-2621.

Stone, J.K., Polishook, J.D., White, J.F. (2004). Endophytic fungi. In *Biodiversity of Fungi, Inventory and Monitoring Methods*, Mueller, G.M., Bills, G.F., Foster, M.S., Eds. Burlington, Elsevier Academic Press, pp 241-270.

Sun, X., Ding, Q., Hyde, K.D., Guo, L.D. (2012). Community structure and preference of endophytic fungi of three woody plants in a mixed forest. *Fungal Ecology* 5:624-632.

Sun, X., Guo, L.-D., Hyde, K.D. (2011). Community composition of endophytic fungi in *Acer truncatum* and their role in decomposition. *Fungal Diversity* 47:85-95.

Suryanarayanan, T.S. (2013). Endophyte research: going beyond isolation and metabolite documentation. *Fungal Ecology* 6:561-568.

Suryanarayanan, T.S., Senthilarasu, G., Muruganandam, V. (2000). Endophytic fungi from *Cuscuta reflexa* and its host plants. *Fungal Diversity* 4:117-123.

Swenson, N.G., Enquist, B.J., Pither, J., Thompson, J., Zimmerman, J. K. (2006). The problem and promise of scale dependancy in community phylogenetics. *Ecology* 87: 2418-2424.

Tadych, M., Ambrose, K.V., Bergen, M.S., Belanger, F.C., White, J.F., Jr. (2012). Taxonomic placement of *Epichloe poae* sp. nov. and horizontal dissemination to seedlings via conidia. *Fungal Diversity* 54:117-131.

Tadych, M., Bergen, M.S., Johnson-Cicalese, J., Polashock, J.J., Vorsa, N., White, J. F. (2012). Endophytic and pathogenic fungi of developing cranberry ovaries from flower to mature fruit: diversity and succession. *Fungal Diversity* 54:101-116.

Taylor, T.N., Hass, H., Kerp, H., Krings, M., Hanlin , R.T. (2005). Perithecial ascomycetes from the 400 million year old Rhynie chert: an example of ancestral polymorphism. *Mycologia* 97:269-285.

Taylor, T.N., Osborn, J.M. (1996). The importance of fungi in shaping the paleoecosystem. *Review of Palaeobotany and Palynology* 90:249-262.

Thomas, M.R., Shattock, R.C. (1986). Filamentous fungal associations in the phylloplane of *Lolium perenne*. *Transactions of the British Mycological Society* 87:255-268.

Udayanga, D., Liu, X., McKenzie, E.H., Chukeatirote, E., Bahkali, A.H., Hyde, K.D. (2011). The genus *Phomopsis*: biology, applications, species concepts and names of common phytopathogens. *Fungal Diversity* 50:189-225.

Unterseher, M. (2011). Diversity of fungal endophytes in temperate forest trees. In *Endophytes of Forest Trees: Biology and Applications,* Pirttila, A.M., Frank, A.C., Eds., accessed online (doi:10.1007/978-94-007-1599-8_2.), Springer Netherlands, pp. 31-46.

Unterseher, M., Persoh, D., Schnittler, M. (2013). Leaf-inhabiting endophytic fungi of European Beech (*Fagus sylvatica* L.) co-occur in leaf litter but are rare on decaying wood of the same host. *Fungal Diversity* 60:43-54.

Unterseher, M., Reiher, A., Finstermeier, K., Otto, P., Morawetz, W. (2007). Species richness and distribution patterns of leaf-inhabiting endophytic fungi in a temperate forest canopy. *Mycological Progress* 6:201-212.

Vamosi, S.M., Heard, S.B., Vamosi, J.C., Webb, C.O. (2009). Emerging patterns in the comparative analysis of phylogenetic community structure. *Molecular Ecology* 18: 572-592.

Wang, Y., Guo, L.D. (2007). A comparative study of endophytic fungi in needles, bark, and xylem of *Pinus tabulaeformis*. *Canadian Journal of Botany* 85:171-184.

Watson, E.S., McClurkin, D.C., Huneycutt, M.B. (1974). Fungal succession on loblolly pine and upland hardwood foliage and litter in North Mississippi. *Ecology* 55:1128-1134.

Wearn, J.A., Sutton, B.C., Morley, N.J., Gange, A.C. (2012). Species and organ specificity of fungal endophytes in herbaceous grassland plants. *Journal of Ecology* 100:1085-1092.

Webb, C.O. (2000). Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. *The American Naturalist* 156: 145-155.

Webb, C.O., Ackerly, D.D., McPeek, M.A., Donoghue, M.J. (2002). Phylogenies and community ecology. *Annual Review of Ecology and Systematics* 33: 475-505.

Weber, R.W., Anke, H. (2006). Effects of endophytes on colonization by leaf surface microbiota. In *Microbial ecology of aerial plant surfaces,* Bailey, M.J., Lilley, A.K., Timms-Wilson, T.M., Spencer-Phillips, P.T., Eds., Cambridge, CABI, pp. 209-222.

Whittaker, R.H. (1965). Dominance and diversity in land plant communities. *Science* 147:250-260.

Wildman, H.G., Parkinson, D. (1979). Microfungal succession on living leaves of Populus tremuloides. *Canadian Journal of Botany* 57:2800-2811.

Wink, M. (2013). Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry* 64:3-19.

Yan, J.F., Broughton, S.J., Yang, S.L., Gange, A.C. (2015). Do endophytic fungi grow through their hosts systemically? *Fungal Ecology* 13:53-59.

Yang, T., Dai, C.-C. (2013). Interactions of two endophytic fungi colonizing *Atractylodes lancea* and effects on the host's essential oils. *Acta Ecologica Sinica* 33:87-93.

Yuan, Z. L., Zhang, C.L., Lin, F.C. (2010). Role of diverse non-systemic fungal endophytes in plant performance and response to stress: progress and approaches. *Journal of Plant Growth Regulation* 29:116-126.

Zambell, C.B., White, J.F. (2014). In the forest vine *Smilax rotundifolia*, fungal epiphytes show site-wide spatial correlation, while endophytes show evidence of niche partitioning. *Fungal Diversity*. doi:10.1007/s13225-014-0316-3

Appendices

Damm, U., Cannon, P.F., Woudenberg, J.H.C., Crous, P.W. (2012). The *Colletotrichum acutatum* species complex. *Studies in Mycology* 73: 37-113.

Glienke, C., Pereira, O. L., Stringari, D., Fabris, J., Kava-Cordeiro, V., Galli-Terasawa, L., Cunnington, J., Shiva, R.G., Groenewald, J.Z., Crous, P.W (2011). Endophytic and pathogenic *Phyllosticta* species, with reference to those associated with Citrus Black Spot. *Persoonia* 26:47-56.

Gomes, R.R., Glienke, C., Videira, S.I.R., Lombard, L., Groenewald, J.Z., Crous, P.W. (2013). *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. *Persoonia* 31: 1-41.

Maharachchikumbura, S.S.N., Guo, L.D., Cai, L., Chukeatirote, E., Wu, W.P., Sun, X., Crous, P.W., Bhat, D.J., McKenzie, E.H.C., Bahkali, A.H., Hyde, K.D. (2012). A multi-locus backbone tree for *Pestalotiopsis* with a polyphasic characterization of 14 new species. *Fungal Diversity*ity 56: 95-129.

Su, Y.Y., Cai, L. (2012). Polyphasic characterisation of three new *Phyllosticta* spp. *Persoonia* 28: 76-84.

Taylor, J.W., Jacobson, D.J., Kroken, S., Kasuga, T., Geiser, D.M., Hibbett, D.S., Fisher, M.C. (2000). Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology* 31: 21-32.

Zhang, K., Su, Y.Y., Cai, L. (2013a). Morphological and phylogenetic characterisation of two new species of *Phyllosticta* from China. *Myological Progress* 12:547-556.

Zhang, K., Zhang, N., Cai, L. (2013b). Typification and phylogenetic study of *Phyllosticta ampelicida* and *P. vaccinii*. *Mycologia* 105: 1030-1042.

APPENDICES

Appendix A: Full spp. abundance tables from winter and summer sampling studies

Table A-1. Winter study (Ch. III), stem endophytic isolates, unabridged table

Sp. Code	Rank	Form	Description	Abund.	Freq.	Prop.
Ph1	1	coelomycete	**"Phyllosticta sp. 1"**	76	48%	17.0%
Pho1	2	coelomycete	**"Phomopsis sp. 1"**	62	39%	13.9%
Co1	3	coelomycete	**"Colletotrichum boninense sensu lato sp. 1"**	56	35%	12.6%
Au	4	hyphomycete	**"Aureobasidium pullulans"**	49	31%	11.0%
My1	5	mycelia sterilia	**"Mycelia sterilia A-Tan"**	46	29%	10.3%
Pho2	6	coelomycete	**"Phomopsis sp. 2"**	22	14%	4.9%
R1	7	coelomycete	**"Colletotrichum acutatum sensu lato sp."**	15	9%	3.4%
G1	8	coelomycete	**"Botryosphaeria 1"**	15	9%	3.4%
M5	9	coelomycete	**"Coniothyrium-like sp. 3"**	8	5%	1.8%
Ph3	10	coelomycete	**"Phyllosticta sp. 2"**	8	5%	1.8%
M7	11	coelomycete	**"Phoma-like sp. 1"**	8	5%	1.8%
M4	12	coelomycete	**"Coniothyrium-like sp. 2A"** (spherical conidia)	8	5%	1.8%
MyY	13	mycelia sterilia	**"Mycelia sterilia A-Yellow"**	7	4%	1.6%
Cld	14	hyphomycete	**"Cladosporium cladosporiodes"**	6	4%	1.3%
Pho3	15	coelomycete	Phomopsis sp 3	5	3%	1.1%
W3	16	mycelia sterilia	**"Mycelia Sterilia C"** (same as "C-White" in summer study)	5	3%	1.1%
M25	17	hyphomycete	Hyphomycete (leaf spot pathogen?)	4	3%	0.9%
Pe4	18	coelomycete	**"Pestalotiopsis sp. 1"**	3	2%	0.7%
M1A	19	coelomycete	**"Coniothyrium-like sp. 2B"** (spherical conidia)	3	2%	0.7%
G4	20	hyphomycete	Alternaria sp.	3	2%	0.7%
R2	21	hyphomycete	Fusarium sp.	3	2%	0.7%
Pe1L	22	coelomycete	**"Pestalotiopsis sp. 2A"**	2	1%	0.4%
M9	23	mycelia sterilia	Mycelia sterilia sp., 'puffballs' in colony	2	1%	0.4%

M20	24	mycelia sterilia	Mycelia sterilia sp.	2	1%	0.4%
M15	25	mycelia sterilia	Mycelia sterilia sp.	2	1%	0.4%
M1Z	26	coelomycete	Phoma sp.	1	1%	0.2%
Sia8	27	hyphomycete	Epicoccum sp.	1	1%	0.2%
Sia6	28	hyphomycete		1	1%	0.2%
Si37	29	mycelia sterilia	Mycelia sterilia sp.	1	1%	0.2%
Si35	30	coelomycete	Colletotrichum (like) sp. 3	1	1%	0.2%
M14	31	mycelia sterilia	Mycelia sterilia sp.	1	1%	0.2%
Stm	32	coelomycete	Stemphylium sp.	1	1%	0.2%
M1C	33	hyphomycete	**Phoma-like sp. 2** (pink spore mass)	1	1%	0.2%
Si32	34	coelomycete	Colletotrichum sp. (probably)	1	1%	0.2%
M1B	35	coelomycete	Coniothyrium sp. 1	1	1%	0.2%
M1D	36	coelomycete	Coniothyrium sp. 2	1	1%	0.2%
M26	37	mycelia sterilia	Mycelia sterilia sp.	1	1%	0.2%
W2	38	mycelia sterilia	**"Mycelia sterilia B"** (same as summer study, Anthostomella)	1	1%	0.2%
Co3	39	coelomycete	may be Colletotrichum morphotype	1	1%	0.2%
Si29	40	mycelia sterilia	phoma like (similar to M7)	1	1%	0.2%
Si30	41	coelomycete	possible colletotrichum sp.	1	1%	0.2%
Si10	42	coelomycete	possible phomopsis (looks like phomopsis beta conidia)	1	1%	0.2%
Si12	43	mycelia sterilia		1	1%	0.2%
Si14	44	mycelia sterilia		1	1%	0.2%
Si15	45	mycelia sterilia		1	1%	0.2%
Si17	46	hyphomycete	Curvularia sp.	1	1%	0.2%
Si22	47	hyphomycete		1	1%	0.2%
M29	48	coelomycete	septate coelomycete	1	1%	0.2%
Co2	49	coelomycete	colletotrichum (maybe) sp.	1	1%	0.2%
Sia4	50	hyphomycete	Curvularia sp.	1	1%	0.2%
M19	51	mycelia sterilia		1	1%	0.2%
			Total Abund.:	446		
			Total Spp.:	51		

Table A-2. Winter study (Ch. III), stem epiphytic isolates, unabridged table

Sp. Code	Rank	Form	Description	Abund.	Freq.	Prop.
Au	1	hyphomycete	**"Aureobasidium pullulans"**	80	50%	11.3%
Pe4	2	coelomycete	**"Pestalotiopsis sp. 1"**	63	39%	8.9%

Pe1L	3	hyphomycete	**"Pestalotiopsis sp. 2A"**	59	37%	8.3%
Cld	4	hyphomycete	**"Cladosporium cladosporiodes"**	59	37%	8.3%
M1B	5	coelomycete	**"Coniothyrium-like sp. 1A"**	49	31%	6.9%
M1D	6	coelomycete	**"Coniothyrium-like sp. 1B"**	44	28%	6.2%
M7	7	coelomycete	**"Phoma-like sp. 1"**	43	27%	6.0%
Pe1D	8	coelomycete	**"Pestalotiopsis sp. 2B"**	37	23%	5.2%
Tr	9	hyphomycete	**"Tripospermum myrti"**	34	21%	4.8%
M4	10	coelomycete	**"Coniothyrium-like sp. 2A"**	28	18%	3.9%
RY	11	Yeast	**"Sporobolomyces sp. (Red Yeast sp.)"**	24	15%	3.4%
M5	12	coelomycete	**Coniothyrium-like sp. 3**	22	14%	3.1%
M1A	13	coelomycete	**Coniothyrium-like sp. 2B**	18	11%	2.5%
M1C	14	coelomycete	**Phoma-like sp. 2**	14	9%	2.0%
W3	15	mycelia sterilia	**"Mycelia sterilia C"**	13	8%	1.8%
M26	16	mycelia sterilia	**"Mycelia sterilia D"**	10	6%	1.4%
W2	17	mycelia sterilia	**"Myclia sterilia B"** (Anthostomella)	10	6%	1.4%
G2	18	coelomycete	**"Diplodia sp. (Botryosphaeria sp. 2)"**	9	6%	1.3%
My1	19	mycelia sterilia	**"Mycelia sterilia A-Tan"**	9	6%	1.3%
M1W	20	coelomycete	**"Ascochyta-like sp. 1"**	8	5%	1.1%
G4	21	hyphomycete	Alternaria sp.	6	4%	0.8%
Pe2	22	coelomycete	Pestalotiopsis sp. 3	4	3%	0.6%
G1	23	coelomycete	**"Botryosphaeria sp. 1"**	4	3%	0.6%
M30	24	coelomycete	pill shaped, aseptate conidia	3	2%	0.4%
M32	25	hyphomycete	similar to Hy1, but different growth on water agar	3	2%	0.4%
Pn1	26	hyphomycete	Penicillium sp.	3	2%	0.4%
M14	27	coelomycete	pill shaped, 1 septate	3	2%	0.4%
R2	28	hyphomycete	Fusarium sp.	3	2%	0.4%
MyY	29	mycelia sterilia	Mycelia sterilia sp.	3	2%	0.4%
M8	30	coelomycete	roundish conidia	2	1%	0.3%
M1H	31	coelomycete	Phoma sp.	2	1%	0.3%
M18	32	hyphomycete	Trichoderma sp.	2	1%	0.3%
M1Z	33	coelomycete	Phoma sp.	2	1%	0.3%
M9	34	mycelia sterilia	puffballs, and no conidia	2	1%	0.3%
Pho2	35	coelomycete	Phomopsis sp. 2	2	1%	0.3%
Stm	36	hyphomycete	Stemphylium sp.	2	1%	0.3%
M22	37	coelomycete	Phoma like sp.	2	1%	0.3%
M11	38	coelomycete	Phoma sp	2	1%	0.3%
Hy1	39	hyphomycete	dark, slow growing hyphomycete	2	1%	0.3%
Si33	40	hyphomycete	unknown hyphomycete	1	1%	0.1%
M21	41	mycelia sterilia	Mycelia sterilia sp.	1	1%	0.1%
Sia7	42	mycelia sterilia	Mycelia sterilia sp.	1	1%	0.1%

Si32	43	coelomycete	Colletotrichum appearing conidia	1	1%	0.1%
M15	44	mycelia sterilia	Mycelia sterilia sp.	1	1%	0.1%
Si25	45	coelomycete	unknown coeolomycete (large, "spotted," conidia"	1	1%	0.1%
Si26	46	mycelia sterilia	Mycelia sterilia sp.	1	1%	0.1%
Si27	47	mycelia sterilia	Mycelia sterilia sp.	1	1%	0.1%
Si31	48	mycelia sterilia	Mycelia sterilia sp.	1	1%	0.1%
Sia9	49	coelomycete	Phoma sp.	1	1%	0.1%
Si11	50	mycelia sterilia	Mycelia sterilia sp.	1	1%	0.1%
Si13	51	hyphomycete	Hemibeltrania sp.	1	1%	0.1%
Si16	52	hyphomycete	possible oomycete	1	1%	0.1%
Si18	53	coelomycete	Phoma or phoma like sp.	1	1%	0.1%
Si19	54	hyphomycete	orange color, produce phialides, not Au	1	1%	0.1%
Si20	55	unclassified	source of conidia/spermatia unknown	1	1%	0.1%
Sia1	56	hyphomycete	maybe Paecilomyces	1	1%	0.1%
Sia5	57	hyphomycete	wheels, arthrinium like	1	1%	0.1%
Si23	58	hyphomycete	Curvularia-like	1	1%	0.1%
Pn2	59	hyphomycete	Penicillium sp.	1	1%	0.1%
Pn3	60	hyphomycete	Penicillium sp.	1	1%	0.1%
Pn4	61	hyphomycete	Penicillium sp.	1	1%	0.1%
Pn5	62	hyphomycete	Penicillium sp.	1	1%	0.1%
Pn6	63	hyphomycete	Penicillium sp.	1	1%	0.1%
Pn7	64	hyphomycete	Penicillium sp.	1	1%	0.1%
M33	65	unclassified	produced chlamydospores	1	1%	0.1%
			Total Abund.:	711		
			Total Spp.:	65		

Table A-3. Winter study (Ch. III), combined surface and interior morphotypes abundance table

(only 1 abundance count allowed per 1-cm segment (1=present, 0=absent) even a morphotype is present on both surface and interior of the same segment; 160 counts possible)

Sp. Code	Rank	Abund.	Freq.	Prop.
Au	1	96	60%	8.7%
Ph1	2	76	48%	6.9%
Pe4	3	63	39%	5.7%
Pho1	4	62	39%	5.6%
Pe1L	5	61	38%	5.5%
Cld	6	60	38%	5.4%
Co1	7	56	35%	5.1%
My1	8	53	33%	4.8%

M7	9	51	32%	4.6%
M1B	10	50	31%	4.5%
M1D	11	45	28%	4.1%
Pe1D	12	37	23%	3.3%
Tr	13	34	21%	3.1%
M4	14	30	19%	2.7%
M5	15	29	18%	2.6%
Pho2	16	25	16%	2.3%
RY	17	24	15%	2.2%
M1A	18	19	12%	1.7%
G1	19	18	11%	1.6%
W3	20	18	11%	1.6%
M1C	21	16	10%	1.4%
R1	22	15	9%	1.4%
M26	23	11	7%	1.0%
W2	24	11	7%	1.0%
G4	25	10	6%	0.9%
MyY	26	10	6%	0.9%
G2	27	9	6%	0.8%
Ph3	28	8	5%	0.7%
M1W	29	8	5%	0.7%
Pho3	30	5	3%	0.5%
M9	31	4	3%	0.4%
Pe2	32	4	3%	0.4%
M14	33	4	3%	0.4%
M25	34	4	3%	0.4%
R2	35	4	3%	0.4%
M30	36	3	2%	0.3%
M32	37	3	2%	0.3%
M1Z	38	3	2%	0.3%
Pn1	39	3	2%	0.3%
Stm	40	3	2%	0.3%
M15	41	3	2%	0.3%
M8	42	2	1%	0.2%
M1H	43	2	1%	0.2%
M18	44	2	1%	0.2%
M20	45	2	1%	0.2%
M22	46	2	1%	0.2%
Si32	47	2	1%	0.2%
M11	48	2	1%	0.2%
Hy1	49	2	1%	0.2%
Si33	50	1	1%	0.1%
Sia8	51	1	1%	0.1%
Sia6	52	1	1%	0.1%
Si37	53	1	1%	0.1%
Si35	54	1	1%	0.1%
M21	55	1	1%	0.1%
Sia7	56	1	1%	0.1%
Co3	57	1	1%	0.1%
Si25	58	1	1%	0.1%
Si26	59	1	1%	0.1%
Si27	60	1	1%	0.1%
Si29	61	1	1%	0.1%

Si30	62	1	1%	0.1%
Si31	63	1	1%	0.1%
Sia9	64	1	1%	0.1%
Si10	65	1	1%	0.1%
Si11	66	1	1%	0.1%
Si12	67	1	1%	0.1%
Si13	68	1	1%	0.1%
Si14	69	1	1%	0.1%
Si15	70	1	1%	0.1%
Si16	71	1	1%	0.1%
Si17	72	1	1%	0.1%
Si18	73	1	1%	0.1%
Si19	74	1	1%	0.1%
Si20	75	1	1%	0.1%
Si22	76	1	1%	0.1%
M29	77	1	1%	0.1%
Co2	78	1	1%	0.1%
Sia1	79	1	1%	0.1%
Sia4	80	1	1%	0.1%
Sia5	81	1	1%	0.1%
Si23	82	1	1%	0.1%
Pn2	83	1	1%	0.1%
Pn3	84	1	1%	0.1%
Pn4	85	1	1%	0.1%
Pn5	86	1	1%	0.1%
Pn6	87	1	1%	0.1%
Pn7	88	1	1%	0.1%
M19	89	1	1%	0.1%
M33	90	1	1%	0.1%
	Total Abund.:	1105		
	Total Spp.:	90		

Table A-4. Summer study (Ch. IV), guide to spp. codes

(names bolded in quotes are used in chapter IV as morphotype names)

Sp. Code	Description or name
Alt	**"Alternaria sp."**
Asp1	Aspergillus sp. 1
Au	**"Aureobasidium pullulans"**
Bot	**"Botryosphaeria spp." - could be several similar spp.**
Cld	**"Cladosporium cladosporiodes"**
Co1	Colletotrichum boninense sp. (similar spores, but different colonies from that of the winter study)
Coel1	coelomycete sp.
Coel2	M4-like coelomycete (like M4 from winter study)
Coel3	chaetomium sp. 1
Coel5	Coelomycete sp.
Coel6	chaetomium sp. 2

Coel7	Phoma-like, pycnidia have hairs
Coel8	a phoma sp.
CR1	dark crust-forming hyphomycete
CR3	crust-forming mycelia sterilia
CR4	crust-forming mycelia sterilia
CR5	crust-forming hyphomycete
CR6	crust-forming hyphomycete
CRM1	**"Dark hyphomycete 2"**
CRM2	Dark, crust forming, hyphomycete
CRM3	dark crust-forming hyphomycete
Dub1	Mycelia sterilia morphotype
Dub3	Mycelia sterilia morphotype
Dub4	Mycelia sterilia morphotype
Dub6	Mycelia sterilia morphotype
Dub7	unknown hyphomycete (conidia only on corn meal agar)
ECC	Epicoccum sp.
HGr	**"Stemphylium sp."**
Hy1	"Dark hyphomycete 1"
Hyp01	Nigrospora sp.
Hyp02	unknown hyphomycete
Hyp03	Curvularia sp.
Hyp05	unknown hyphomycete (yeast like)
Hyp06	Stemphylium sp. 2
Hyp07	unknown hyphomycete
Hyp08	Mycelia sterilia with microscopic crystals
Hyp10	Trichoderma-like sp.
Hyp11	unknown hyphomycete (diamond like conidia)
Hyp12	unknown hyphomycete
Hyp13	hyphomycete, forms pink slime
Hyp14	unknown hyphomycete
M1Cw	Phoma sp.
Mo3	Mycelia sterilia
Mo4	unknown coelomycete
Mo5	unknown hyphomycete
Mo6	Mycelia sterilia
Mo7	crust-forming hyphomycete sp.
MS01	Mycelia sterilia sp.
MS10	Mycelia sterilia morphotype (orange)
MS11	Mycelia sterilia (orange and ringed)
MS12	Coniothyrium-like conidia, red and white colonies
MS13	Mycelia sterilia morphotype
MS2	Mycelia sterilia morphotype

MS3	Mycelia sterilia sp.
MS4	Mycelia sterilia morphotype
MS5	Mycelia sterilia morphotype
MS7	Mycelia sterilia (orange)
My1b	**"Mycelia sterilia A-Tan"**
My1y	**"Mycelia sterilia A-Yellow"**
Pe1D	**"Pestalotiopsis sp. 2B"**
Pe1L	**"Pestalotiopsis sp. 2A"**
Pe2	**"Pestalotiopsis sp. 3"**
Pe4	**"Pestalotiopsis sp. 1"**
Ph1	**"Phyllosticta sp. 1"**
Ph3	**"Phyllosticta sp. 2"**
Pho1	**"Phomopsis sp. 1"**
Pho2	**"Phomopsis sp. 2"** (was commmon in winter study)
Pho4	Phomopsis sp. 4
Pho5	Phomopsis sp. 5
Phoma1	**"Coniothyrium-like morphotype"**
phoma2	phoma sp. (forms gooey mound colony)
PhomaR	Phoma sp. with red hyphae
Pn1	**"Penicillium sp. 1"**
Pn2	Penicillium sp. 2
R1	**"Colletotrichum acutatum"**
sinN01	Phoma-like sp., very small conidia
sinN3	unclassified
sinN4	Mycelia sterilia
sinN5	Mycelia sterilia
SinN6	coelomycete sp.
sinN12	Mycelia sterilia
sinN14	Phoma sp.
sinN21	coelomycete (empty pycnidia with setae)
sinN25	hyphomycete sp.
sinN26	unclassified
Td1	Trichoderma morphotype 1
Td2	Trichoderma morphotype 2
Td3	Trichoderma morphotype 3
Td4	Trichoderma morphotype 4
TRP	Tripospermum myrti
Vert	Verticillium sp.
W2	"Mycelia sterilia B-White" (Anthostomella)
W2p	"Mycelia sterilia E-Pink"
W3	"Mycelia sterilia C-White"

Table A-5. Summer study (Ch. IV), abundances by major sites; unabridged

(all datasets included in single table, I.stem interior, II.leaf interior, III.stem surface, IV.leaf surface)

I. Stem Endophytes

Sp. Code	Rank	PB	SH	RU	All Sites
My1y	1	28	13	18	59
Pho1	2	16	27	6	49
Ph1	3	8	17	18	43
My1b	4	16	4	5	25
W2	5	12	10	1	23
R1	6	0	11	8	19
Au	7	9	6	3	18
Ph3	8	10	7	0	17
W3	9	8	5	3	16
Bot	10	0	3	3	6
Mo3	11	0	0	3	3
Alt	11	0	1	2	3
Cld	13	1	0	1	2
Co1	13	0	1	1	2
Pho2	13	0	1	1	2
Wp2	13	1	1	0	2
SinN6	13	0	1	1	2
Mo4	31	0	0	0	0
Hyp14	18	1	0	0	1
MS11	18	1	0	0	1
sinN21	18	0	0	1	1
sinN25	18	0	0	1	1
sinN26	18	0	0	1	1
Mo5	18	1	0	0	1
sinN4	18	0	1	0	1
sinN5	18	0	0	1	1
sinN12	18	1	0	0	1
Mo6	18	0	0	1	1
Mo7	18	0	1	0	1
sinN01	18	0	0	1	1
sinN3	18	0	1	0	1
	Total Abund.:	113	111	80	304
	Total Spp:	14	18	21	30

II. Leaf Interiors

Sp. Code	Rank	PB	SH	RU	All Sites
Ph3	1	13	10	6	29
Ph1	2	2	11	13	26
W2	3	14	7	4	25
Wp2	4	2	5	0	7
Pho1	5	2	3	1	6
R1	6	0	1	1	2
Mo4	6	1	1	0	2
W3	8	1	0	0	1

My1b	8	0	0	1	1
Pho5	8	0	0	1	1
Au	8	0	0	1	1
sinN14	8	0	0	1	1
Alt	8	0	1	0	1
	Total Abund.:	35	39	29	103
	Total Spp:	7	8	9	13

II. Stem Surfaces

Sp. Code	Rank	PB	SH	RU	All Sites
Au	1	33	43	29	105
Pe1D	2	31	23	22	76
Pe1L	3	37	12	22	71
Pe4	4	28	6	11	45
Cld	5	6	12	15	33
Alt	6	3	11	12	26
W2	7	7	12	3	22
HGr	8	3	3	13	19
Phoma1	9	1	10	6	17
Pe2	10	10	2	0	12
Bot	11	1	2	7	10
Pn1	12	3	4	2	9
M1Cw	13	0	4	3	7
Hyp03	14	2	2	1	5
Td1	14	4	0	1	5
Td3	14	3	0	2	5
TRP	14	0	3	2	5
ECC	14	1	1	3	5
MS7	19	1	2	1	4
CRM2	19	2	0	2	4
Hy1	19	1	1	2	4
Hyp01	19	0	1	3	4
Mo3	23	0	1	2	3
R1	23	0	0	3	3
Hyp10	23	2	1	0	3
CRM1	23	1	1	1	3
W3	27	2	0	0	2
My1y	27	0	0	2	2
MS01	27	0	2	0	2
MS3	27	2	0	0	2
Pn2	27	0	0	2	2
Asp1	27	1	0	1	2
Coel1	27	0	2	0	2
PhomaR	27	2	0	0	2
CRM3	27	2	0	0	2
Wp2	36	0	1	0	1
My1b	36	0	0	1	1
Ph1	36	0	0	1	1
Hyp02	36	1	0	0	1
Hyp05	36	0	0	1	1
Hyp06	36	0	0	1	1
Hyp08	36	1	0	0	1

Hyp11	36	1	0	0	1
Hyp12	36	1	0	0	1
Dub1	36	0	0	1	1
Dub3	36	1	0	0	1
Dub4	36	0	1	0	1
Dub6	36	1	0	0	1
Dub7	36	0	0	1	1
MS2	36	0	1	0	1
MS4	36	0	1	0	1
MS5	36	0	1	0	1
MS10	36	1	0	0	1
MS12	36	0	0	1	1
MS13	36	0	0	1	1
CR3	36	1	0	0	1
CR4	36	0	0	1	1
Coel2	36	0	0	1	1
Coel3	36	0	0	1	1
Coel5	36	1	0	0	1
Coel6	36	0	0	1	1
Coel8	36	0	0	1	1
phoma2	36	0	1	0	1
Td2	36	0	0	1	1
Td4	36	1	0	0	1
Vert	36	0	0	1	1
	Total Abund.:	199	167	188	554
	Total Spp:	36	30	42	66

IV. Leaf Surfaces

Sp. Code	Rank	PB	SH	RU	All Sites
Au	1	12	8	11	31
W2	2	10	6	3	19
Hy1	3	6	1	2	9
Cld	4	0	3	4	7
CRM1	5	5	1	0	6
Alt	6	2	1	2	5
Pe1D	6	1	0	4	5
Phoma1	8	0	1	3	4
TRP	9	0	2	1	3
Pe1L	9	0	1	2	3
CR1	11	1	1	0	2
R1	11	1	1	0	2
W3	11	2	0	0	2
Pe4	11	0	0	2	2
Hyp01	11	1	1	0	2
Bot	11	0	0	2	2
Asp1	11	0	1	1	2
CRM2	18	1	0	0	1
Hyp07	18	0	0	1	1
Hyp13	18	1	0	0	1
Coel7	18	0	0	1	1
Mo5	18	1	0	0	1

Pho2	18	0	0	1	1
My1y	18	0	0	1	1
CR5	18	1	0	0	1
CR6	18	0	1	0	1
Coel5	18	1	0	0	1
M1Cw	18	0	1	0	1
PhomaR	18	0	1	0	1
CRM3	18	1	0	0	1
Wp2	18	0	1	0	1
HGr	18	0	1	0	1
Ecc	18	0	1	0	1
Pho4	18	0	0	1	1
	Total Abund.:	47	34	42	123
	Total Spp:	16	19	17	34

Appendix B: Stem vs. leaf comparisons (summer study, Ch. IV)

Table A-6. Stems vs. leaves: comparing endophytes across tissues

Sp. Code	Stem-Abund.	Leaf-Abund.	Stem-Freq.	Leaf-Freq.	Stem-Prop.	Leaf-Prop.
My1y	59	0	36%	0%	19%	0%
Pho1	49	6	30%	11%	16%	6%
Ph1	43	26	27%	48%	14%	25%
My1b	25	1	15%	2%	8%	1%
W2	23	25	14%	46%	8%	24%
R1	19	2	12%	4%	6%	2%
Au	18	1	11%	2%	6%	1%
Ph3	17	29	10%	54%	6%	28%
W3	16	1	10%	2%	5%	1%
Bot	6	0	4%	0%	2%	0%
Mo3	3	0	2%	0%	1%	0%
Alt	3	1	2%	2%	1%	1%
Cld	2	0	1%	0%	1%	0%
Co1	2	0	1%	0%	1%	0%
Pho2	2	0	1%	0%	1%	0%
Wp2	2	7	1%	13%	1%	7%
SinN6	2	0	1%	0%	1%	0%
Mo4	0	2	0%	4%	0%	2%
Hyp14	1	0	1%	0%	0%	0%
MS11	1	0	1%	0%	0%	0%
sinN21	1	0	1%	0%	0%	0%
sinN25	1	0	1%	0%	0%	0%
sinN26	1	0	1%	0%	0%	0%
Mo5	1	0	1%	0%	0%	0%
sinN4	1	0	1%	0%	0%	0%
sinN5	1	0	1%	0%	0%	0%
sinN12	1	0	1%	0%	0%	0%
Mo6	1	0	1%	0%	0%	0%
Mo7	1	0	1%	0%	0%	0%
sinN01	1	0	1%	0%	0%	0%
sinN3	1	0	1%	0%	0%	0%
Pho5	0	1	0%	2%	0%	1%
sinN14	0	1	0%	2%	0%	1%

Total Abund.:	304	103
Total Spp:	30	13

Table A-7. Stems vs. leaves: comparing epiphytes across tissues

Sp. Code	Stem-Abund.	Leaf-Abund.	Stem-Freq.	Leaf-Freq.	Stem-Prop.	Leaf-Prop.
Au	105	31	65%	57%	19%	24%
Pe1D	76	5	47%	9%	14%	4%
Pe1L	71	3	44%	6%	13%	2%
Pe4	45	2	28%	4%	8%	2%
Cld	33	7	20%	13%	6%	5%
Alt	26	5	16%	9%	5%	4%
W2	22	19	14%	35%	4%	15%
HGr	19	1	12%	2%	3%	1%
Phoma1	17	4	10%	7%	3%	3%
Pe2	12	0	7%	0%	2%	0%
Bot	10	2	6%	4%	2%	2%
Pn1	9	0	6%	0%	2%	0%
M1Cw	7	1	4%	2%	1%	1%
Hyp03	5	0	3%	0%	1%	0%
Td1	5	0	3%	0%	1%	0%
Td3	5	0	3%	0%	1%	0%
TRP	5	3	3%	6%	1%	2%
ECC	5	1	3%	2%	1%	1%
MS7	4	0	2%	0%	1%	0%
CRM2	4	9	2%	17%	1%	7%
Hy1	4	9	2%	17%	1%	7%
Hyp01	4	2	2%	4%	1%	2%
Mo3	3	0	2%	0%	1%	0%
R1	3	2	2%	4%	1%	2%
Hyp10	3	0	2%	0%	1%	0%
CRM1	3	6	2%	11%	1%	5%
W3	2	2	1%	4%	0%	2%
My1y	2	1	1%	2%	0%	1%
MS01	2	0	1%	0%	0%	0%
MS3	2	0	1%	0%	0%	0%
Pn2	2	0	1%	0%	0%	0%
Asp1	2	2	1%	4%	0%	2%
Coel1	2	0	1%	0%	0%	0%
PhomaR	2	1	1%	2%	0%	1%
CRM3	2	1	1%	2%	0%	1%
CR1	0	2	0%	4%	0%	2%
Coel5	1	1	1%	2%	0%	1%

Wp2	1	1	1%	2%	0%	1%
Pho2	0	1	0%	2%	0%	1%
Hyp02	1	0	1%	0%	0%	0%
Hyp05	1	0	1%	0%	0%	0%
Hyp06	1	0	1%	0%	0%	0%
Hyp08	1	0	1%	0%	0%	0%
Hyp11	1	0	1%	0%	0%	0%
Hyp12	1	0	1%	0%	0%	0%
Dub1	1	0	1%	0%	0%	0%
Dub3	1	0	1%	0%	0%	0%
Dub4	1	0	1%	0%	0%	0%
Dub6	1	0	1%	0%	0%	0%
Dub7	1	0	1%	0%	0%	0%
MS2	1	0	1%	0%	0%	0%
MS4	1	0	1%	0%	0%	0%
MS5	1	0	1%	0%	0%	0%
MS10	1	0	1%	0%	0%	0%
MS12	1	0	1%	0%	0%	0%
MS13	1	0	1%	0%	0%	0%
CR3	1	0	1%	0%	0%	0%
CR4	1	0	1%	0%	0%	0%
Coel2	1	0	1%	0%	0%	0%
Coel3	1	0	1%	0%	0%	0%
Coel6	1	0	1%	0%	0%	0%
Coel8	1	0	1%	0%	0%	0%
phoma2	1	0	1%	0%	0%	0%
Td2	1	0	1%	0%	0%	0%
Td4	1	0	1%	0%	0%	0%
Vert	1	0	1%	0%	0%	0%
Hyp07	0	1	0%	2%	0%	1%
Hyp13	0	1	0%	2%	0%	1%
Coel7	0	1	0%	2%	0%	1%
Mo5	0	1	0%	2%	0%	1%
CR5	0	1	0%	2%	0%	1%
CR6	0	1	0%	2%	0%	1%
Pho4	0	1	0%	2%	0%	1%
My1b	1	0	1%	0%	0%	0%
Ph1	1	0	1%	0%	0%	0%

Total Abund.	554	131
Total Spp:	66	34

Appendix C: R code used for seasonal analysis (Ch. IV)

A reduced dataset was generated from the winter study in which only the three samples near the tip were included for each plant, rather than the total 10 samples per plant. The total presence counts were summed for each plant (so 0 to 3 counts). The final document is a tab delimited text file, in which species names are the top column, and plants 1-16 are listed as the first row. The example below uses the column header "X.Ph1.," referring to morphotype "*Phyllosticta* sp. 1."

```
# First the dataset is read in and attached
SmilaxData<-read.table(location of the reduced dataset file is entered here in quotes)
attach(SmilaxData)
names(SmilaxData)

#here I generate a bootstrap dataset, in which 6 of the 16 stems are chosen, with
replacement (so the same stem can be picked twice), over 10,000 replicates. The presence
counts of species X.Ph1. are summed each time.

sum.x1=NULL
for (i in 1:10,000)
{
   sample(X.Ph1.,replace=T)->x1
   sum.x1[i]=sum(x1[1:6])
}

#Here the bootstrapped results are shown as a histogram, then as quantiles and median.

hist(sum.x1,breaks=x(-0.5 ,0.5 ,1.5 ,2.5 ,3.5 ,4.5 ,5.5 ,6.5 ,7.5 ,8.5 ,9.5 ,10.5 ,11.5 ,12.5
,13.5 ,14.5 ,15.5 ,16.5 ,17.5 ,18.5))
quantile(sum.x1, probs=c(5,95)/100)
median(sum.x1)

#the same program can be repeated then just changing the name of the species from
X.Ph1. to something different.
```

Appendix D: Multi-gene phylogenetic placement of common morphotypes

Isolates

In August of 2011 I sampled epiphytic and endophytic fungi from a total of 54 *S. rotundifolia* plants across three main locations in New Jersey (see chapter IV).

Molecular Procedure

ITS sequences were acquired for all isolates. Sequences of one other selected gene were acquired for each genus based on literature review. For *Pestalotiopsis*, *Phyllosticta*, and *Phomopsis* spp., ITS and TEF1 were used. For *Colletotrichum* ITS and GAPDH were used. The details of these primers are given below:

PRIMERS:

ITS = internal transcribed spacer region of DNA coding for ribosomal RNA, including partial small subunit, ITS1, 5.8S ribosomal gene, ITS2, partial large subunit.
FORWARD = ITS1 = 5'-TCCGTAGGTGAACCTGCGG-3' (White et al. 1990)
REVERSE = ITS4 = 5'-TCCTCCGCTTATTGATATGC-3' (White et al. 1990)

TEF1 = intron of translation elongation factor 1- α gene:
FORWARD = Ef-728M = 5'-CATYGAGAAGTTCGAGAAGG-3' (Samuels et al. 2012, a slight variation of EF1-728f, Carbone and Kohn 1999)
REVERSE = Ef2 = GGARGTACCAGTSATCATGTT-3' (O'Donnell et al. 1998)
(codes: Y = C/T; R= A/G; S = G/C)

GAPDH = intron of glyceraldehyde 3 phosphate dehydrogenase gene:
FORWARD = GDF1 = 5'-GCCGTCAACGACCCCTTCATTGA-3' (Guerber et al. 2003)
REVERSE = GDR1 = 5'-GGGTGGAGTCGTACTTGAGCATG-3' (Guerber et al. 2003)

PCR mix, final volume 50 µl (Made as master mix, 45 ul + 5 ul DNA); see note re: ITS*

Add (µl)	Reagent	Final Concentration
30.10	water (PCR grade)	-
5.00	10X Genscript PCR reaction buffer (500 mM KCl (ph 9), 15 mM $MgCl_2$, 1% Triton X-100 Buffer)	1x (50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100 buffer)
0.50	50 mM $MgCl_2$	0.5 mM $MgCl_2$, (total 2.0 mM including buffer above)
1.00	50 mM dNTP's (each) (50x)	0.2 mM (200 uM)
4.00	Forward Primer (5 uM)	0.4 uM (or pmole/ul)
4.00	Reverse Primer (5 uM)	0.4 uM (or pmole/ul)
0.40	Genscript Taq DNA Polymerase (5 units/µl)	1 unit per reaction
Scale up desired no. of reactions (multiply above volumes by x 1.1) and combine above ingredients to form master mix.		
5.0 µl (add to 45 µl master mix)	Template DNA (10 ng/µl)	1 ng/ µl

*exactly as shown for TEF1, GAPDH. For ITS1+ITS4, the primer volumes were typically slightly lower, at 0.3 uM final concentration, and in one instance Bovine Serum Albumin was added, at a final concentration of 0.4 ug/ul to facilitate binding of Taq in extraction samples with impurities.

PCR PROGRAMS:

Gene: ITS

(0) preheat lid 100°C

(1) 95°C, 2 (-5) min *initial denaturing step*

(2) 95°C, 30 sec *denature*
(3) 57°C, 57 sec *anneal*
(4) 72°C, 57 sec *extend*

loop (4)->(2) 39 times (40 total cycles)

(5) 72°C, 7 (-10) min *final extension*
(6) 10°C, pause *preserve*

Gene: TEF1

(0) preheat lid, 100°C

(1) 94°C, 2 min *initial denaturing step*

(2) 94°C, 30 sec *denature*
(3) 65°C, 30 sec *anneal* (-1°C each progressive cycle)
(4) 72°C, 60 sec *extend*

loop 4 ->2, 15 times (16 total cycles)

(5) 94°C, 30 sec *denature*
(6) 48°C, 30 sec *anneal*
(7) 72°C, 60 sec *extend*

loop 7->5 34 times (35 total cycles)

(8) 72°C, 10 min *final extension*
(9) 10°C, pause *preserve*

(from Samuels and Ismaiel, 2009)

GAPDH (from Damm et al. 2012):

(0) preheat lid 100°C

(1) 94°C, 5 min *initial denaturing step*

(2) 94°C, 30 sec *denature*
(3) 52°C, 30 sec *anneal*
(4) 72°C, 30 sec *extend*

loop (4)->(2) 39 times (40 total cycles)

(5) 72°C, 7 min *final extension*
(6) 10°C, pause *preserve*

(from Damm et al. 2012)

Alignment: Reference alignments were taken from treeBASE (treebase.org) or sequences were directly downloaded from Genbank based on accession numbers given in the literature. After adding my own sequences alignment was done using MAFFT (G-INS-I algorithm) followed by minor manual adjustments, or in some cases manual addition of my sequences to the previous treeBASE derived alignment.

Neighbor Joining: Neighbor joining trees were generated for each individual gene using Mega 5.2.1 with 10,000 bootstrap replications under the conditions, "Method: p-distance", "Substitutions to Include: d: Transitions + Transversions", and "Gaps/Missing Data Treatment: Pairwise Deletion."

Bayesian phylogeny: Each gene alignment was then tested for optimum model in jModelTest v2.1.4 using 3 possible nucleotide substitution schemes, and the final model chosen according to AICc. MrBayes v3.2.1 was used to apply separate models to each gene, and then run per defaults for 3 million to 6 million generations until standard deviation of split frequencies was <0.01, and 25% of trees were discarded as burn-in before the final tree was constructed. Trees were edited in FigTree v1.4.0 and Serif DrawPlus.

Results

***Pestalotiopsis*:**

Tree shown in chapter 5, fig. V-1.

Neighbor Joining Results: ITS – '*Pestalotiopsis* sp. 2B" clusters with 82% bootstrap support, sp. "*Pestalotiopsis* sp. 1" clusters with 87% support, while "*Pestalotiopsis* sp. 2A" and '*Pestalotiopsis* sp. 3' are not monophyletic. Tef1 – '*Pestalotiopsis* sp. 2B' clusters with 99% support, 'Pestalotiopsis sp. 2A' clusters with 89% support, '*Pestalotiopsis* sp. 1' clusters with 100% support, and '*Pestalotiopsis* sp. 3' with 100% support.

Discussion: A third gene locus is needed since ITS does a poor job of resolving spp. "3" and "2A".

Phyllosticta:

Tree shown in chapter 5, figure V-11.

Neighbor Joining Results: ITS – '*Phyllosticta* sp. 1' clusters with 99% bootstrap support and '*Phyllosticta* sp. 2' clusters with 93% support. Tef1 - '*Phyllosticta* sp. 1' clusters with 100% support and '*Phyllosticta* sp. 2' clusters with 99% support.

Discussion: These both seem to be solidly supported species. In the Bayesian tree, species "1" is sister to a clade of *Phyllosticta* with coniferous evergreen hosts. Species "3" seems to be closely related to a number of *Phyllosticta* spp. that occupy other evergreen plants, with the exception of the *Fraxinus* species. *S. rotundifolia*'s stem is evergreen and some of the leaves survive through late into the winter.

Phomopsis:

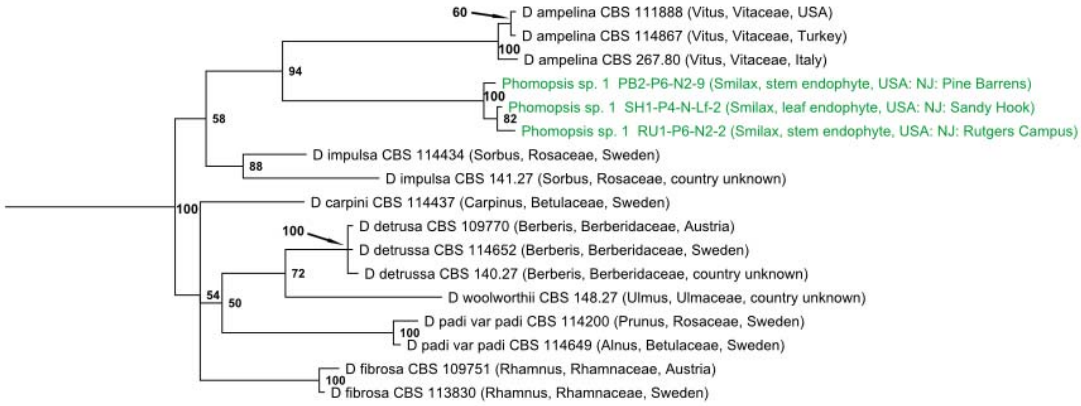

Fig. A-1. Multi-gene placement for *Phomopsis* sp. 1 isolates

Portion of phylogenetic tree from Bayesian analysis of a partitioned alignment of genes ITS (K80+I+G model) and Tef1 (JC model) using my own isolates (indicated in green) and the alignment/dataset of Gomes et al. (2013) downloaded from treeBASE, study 13943. Posterior probabilities are shown at nodes.

Neighbor Joining Results: ITS – *Phompsis* sp. 1 clusters with 99% bootstrap support. Tef1 – Species "1" clusters with 100% bootstrap support.

Discussion: This *Phomopsis* morphotype receives strong support as a monophyletic clade from both genes. Its closest known relative within the *Phomopsis* family tree is a pathogen of grape vines, *D. ampelina,* the current accepted name for

Phomopsis viticola (discussed in Ch.5, as an endophytic pathogen). The rest of the clade contains species from trees of diverse plant families.

***Colletotrichum*:**

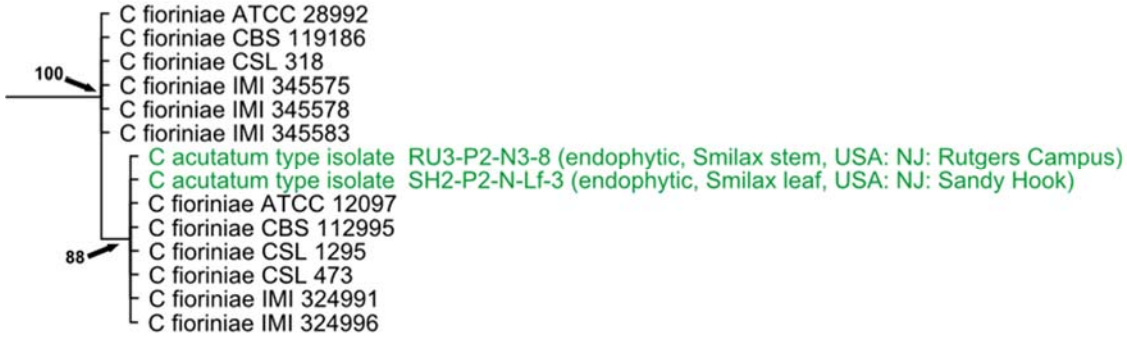

Fig. A-2. Multi-gene placement for *Colletotrichum acutatum* isolates

Portion of phylogenetic tree from Bayesian analysis of a partitioned alignment of genes ITS (K80+I model) and GAPDH (JC model) including my own isolates (indicated in green) and the alignment/ dataset of Damm et al. (2013), downloaded from treeBASE (study 12762), and reduced in size to speed computing times. The ex-type strain of *C. fioriniae* was accidentally removed from th Posterior probabilities are shown at the nodes.

Neighbor Joining Results: *C. acutatum* type isolates were interspersed amongst the *C. fioriniae* clade with bootstrap support of 95% (ITS) and 100% (GAPDH) for the clade as a whole. Note that a third Pine Barrens isolate was included in the ITS dataset.

Discussion: These isolates are members of the species *C. fioriniae*, described by Damm et al. (2012) as a multi-host fruit rot pathogen, an endophyte, and in one instance found as an entomopathogen of scale insects. From this it can be concluded that the isolates are probably not host specific to greenbrier.

Appendix E: Generated nucleotide sequences

(1) *Pestalotiopsis* sp. 1 (sp. code: Pe4)

Pe4-a, or PB3-P3-T3-4
>ITS: Accession no. KP122296

Pe4-b, or SH1-P5-T1-7
>ITS: Accession no. KP 122297
>Tef1 (Pe4-b_SH1-P5-T1-7)
AGAAGGTTGGTCATCCTCACAATTCCTTCATGTCCATCATGGCATTCCATCGAGCACTTGATGCGGTGCCGCGAATGAT
TTTTTTCCCCCTTCACCAGATACCTATCCACATTCTCTCACACTTACCCCGCCGCACGACCCCGCGGTGCAAACGAAAAA
AATTCTTATCAGCCCCACCTCGCACAAACATTTTGGCAGCCACGCACGTTCATGACCCACAATGAGCCATTGCTGACCC
CGCCAAACAGGAAGCCGCCGAGCTCGGAAAGGGTTCCTTCAAGTACGCATGGGTTCTTGACAAGCTCAAGGCCGAGCG
TGAGCGTGGTATCACCATCGATATCGCTCTCTGGAAGTTCGAGACCAACGAGTACAATGTCACCGTCATTGGTTAGTAT
CCCTGTTCACAACAAGCACCATGCATCCGACATCAGACTAACATTGCAATACAGACGCTCCCGGTCACCGTGACTTCAT
CAAGAACAT

Pe4-c, or RU1-P4-T1-4
>ITS: Accession no. KP122298
>Tef1 (Pe4-c_RU1-P4-T1-4)
TTCGAGAAGGTTAGTCATCCTCACAATCCCTTCATGTCCATCATGGCATTCCATCGAGCATTTCACGCGGTGCTGGGAAT
GGTTTTCTTTCCTCGCCAGACACCTGCCCACATTTCCTCACACTTACCCCGCCGCACGACCCCGCGGTGCAAACGAAAA
AAATTCTTATCAGCCCCACCTCGCACAAACATTTTGGCAGCCACGCACGTTCATGACCCACAATGAGCCATTGCTGACC
CCGCCAAACAGGAAGCCGCCGAGCTCGGAAAGGGTTCCTTCAAGTACGCATGGGTTCTTGACAAGCTCAAGGCCGAGC
GTGAGCGTGGTATCACCATCGATATCGCTCTCTGGAAGTTCGAGACCAACGAGTACAATGTCACCGTCATTGGTTAGTA
TCCCTGTTCACAACAAGCACCATGCATCCGAAATCAGACTAACATGGCAATACAGACGCTCCCGGTCACCGTGACTTCA
TCAAGAACATG

Pe4-d or PB2-P5-T2-8
>ITS: Accession no. KP122299
>Tef1 (Pe4d_PB3-P3-T3-4)
GAAGTTCGAGAAGGTTAGTCATCCTCACAATTCCTTCATGTCTATCATGGCATTCCATCGAGCACTTGATGCGGTGCCG
CGAATGGTTTTCCCCCCCTTCACCAGACACCTGCCCACATTCTCTCACACTTACCCCGCCGCACGACCCCGCGGTGCAA
ACGAAAAAAAATTCTTTATCAGCCCCACCTCGCACAAACATTTTGGCAGCCACGCACGTTCATGACCCACAATGAGCCA
TTACTGACCCCGCCAAACAGGAAGCCGCCGAGCTCGGAAAGGGTTCCTTCAAGTACGCATGGGTTCTTGACAAGCTCA
AGGCCGAGCGTGAGCGTGGTATCACCATCGATATCGCTCTCTGGAAGTTCGAGACCAACGAGTACAATGTCACCGTCAT
TGGTTAGTATCCCTGTTCACAACAAGCACCATGCATCCGAAATCAGACTAACATGGCAATACAGACGCTCCCGGTCACC
GTGACTTCATCAA

(2) *Pestalotiopsis* sp. 2A (sp. code: Pe1L)

Pe1L-a, or PB2-P3-T3-3
>ITS: Accession no. KP122288
>Tef1 (Pe1L-a_PB2-P3-T3-3)
TTCGAGAAGGTTAGTCATTTTCAAATCCCATCATTCCCATCCTCATCATCGCCTCGCAAACATTTTCCAACCGGTGCCGA
GAATCTGTTTTCGCACCTGCCCATTTTCCCAGACACTTACCCCGCCGCACGACCCCGCGGTGCAAACGAAAAATTTCTT
ATCACAGCCCCACATCGCACAAACATTTTGGCAGCCGTGCACTTTTCAAGACCCACAATGAACAATTGCTGACCCCGCC
AAATAGGAAGCCGCCGAGCTCGGAAAGGGTTCCTTCAAGTACGCATGGGTTCTTGACAAGCTCAAGGCCGAGCGTGAG
CGTGGTATCACCATCGATATCGCTCTCTGGAAGTTCGAGACCAACGAGTACAATGTCACCGTCATTGGTTAGTATCCCT
GTCCACACGATGTACCATGCATTGAATGTATACTAACATGGCAACACAGATGCTCCCGGTCACCGTGATTTCATCAAGA
ACATG

Pe1L-b, or RU2-P1-T-Lf-3
>ITS: Accession no. KP122289

>Tef1 (Pe1L-b_RU2-P1-T-Lf-3)
TTCGAGAAGGTTAGTCATCCTCAAATCCCATCATTCCCATCCTCATCATCATCGCCTCGCAAACATTTTCCAACCGGTGC
CGAGAATCTGTTTTCGCATCTGCCCATTTTCCCAGACACTTACCCCGCCGCACGACCCCGCGGTGCAAACGAAAAAATT
CTTATCACAGCCCCACATCACACAAACATTTTGGCAGCCATGCACTTTCCAAGACCCACAATGAACATTTGCTGACCCC
GCCAAATAGGAAGCCGCCGAGCTCGGAAAGGGTTCCTTCAAGTACGCATGGGTTCTTGACAAGCTCAAGGCCGAGCGT
GAGCGTGGTATCACCATCGATATCGCTCTCTGGAAGTTCGAGACCAACGAGTACAATGTCACCGTCATTGGTTAGTATC
CCTGTCCACACGATGTACTATGCATCTGAATGTATACTAACATGGCAACACAGATGCTCCCGGTCACCGTGATTTCATC
AAGAACATG

Pe1L-c, or SH2-P2-T3-1
>ITS: Accession no. KP122290

(3) *Pestalotiopsis* sp. 2B (sp. code: Pe1D)

Pe1D-a, or SH1-P1-T2-6
>ITS: Accession no. KP122291
>Tef1 (Pe1D-a_SH1-P1-T2-6)
AGAAGGTTAGTCATCCTCGCAATCCCCATCATTNTCATCTTCACCATCATCACCTCGCAAACATTCCCACATCGGTGCCG
AAAATCTGGATTTCGCACCTGCCCATTTTCCCAAACACTTACCCCGCCGCACGACCCCGCGGTGCAAACGAAAAATTTC
TTATCGCAGCCCCACATCACACAAACATTTTGGCAGCCACGCACTTTGCATGACCCACAATGAACAATTGCTGACCCCG
CCAAATAGGAAGCCGCCGAGCTCGGAAAGGGTTCCTTCAAGTACGCATGGGTTCTTGACAAGCTCAAGGCCGAGCGTG
AGCGTGGTATCACCATTGATATCGCTCTCTGGAAGTTCGAGACCAACGAGTACAATGTCACCGTCATTGGTTAGTATCC
CTGTCCACAACATGTGTCATGTCTCTGAACTCAAGACTAACCTCGCAACACAGACGCTCCCGGTCACCGTGATTTCATC
AAGAACATGA

Pe1D-b, or RU1-P4-T3-3
>ITS: Accession no. KP122292
>Tef1 (Pe1D-b_RU1-P4-T3-3)
AGAAGGTTAGTCATCCTCGCAATCCCATCATCCCCATCCTCATCAACATCACCTCGCAAACATTTCCACACCGGTGCCG
AAAATCTGGTTTCCGCACCTGCCCATTTTCCCAGACACTTACCCCGCCGCACGACCCCGCGGTGCAAACGAAAAATTTC
TTATCGCAGCCCCACATCACAAACGTTTTGGCAGCCACGCACTTTGCATGACCCACAATGAACAATTGCTGACCCCGCC
AAATAGGAAGCCGCCGAGCTCGGAAAGGGTTCCTTCAAGTACGCATGGGTTCTTGACAAGCTCAAGGCCGAGCGTGAG
CGTGGTATCACCATTGATATCGCTCTCTGGAAGTTCGAGACCAACGAGTACAATGTCACCGTCATTGGTTAGTATCCCT
GTCCACAACATGTGTCATGTCACCGAACTCAAGACTAACCTTGCAATACAGACGCTCCCGGTCACCGTGATTTCATCAA
GAACATG

Pe1D-c, or PB3-P2-T3-8
>ITS: Accession no. KP122293
>Tef1 (Pe1Dc_PB3-P2-T3-8)
TTCGAGAAGGTTAGTCATCCTCGCAATCCCCATCATTCTCATCTTCACCATCATCACCTCGCAAACATTCCCACATCGGT
GCCGAAAATCTGGATTTCGCACCTGCCCATTTTCCCAAACACTTACCCCGCCGCACGACCCCGCGGTGCAAACGAAAAA
TTTCTTATCGCAGCCCCACATCACACAAACATTTTGGCAGCCACGCACTTTGCATGACCCACAATGAACAATTGCTGAC
CCCGCCAAATAGGAAGCCGCCGAGCTCGGAAAGGGTTCCTTCAAGTACGCATGGGTTCTTGACAAGCTCAAGGCCGAG
CGTGAGCGTGGTATCACCATTGATATCGCTCTCTGGAAGTTCGAGACCAACGAGTACAATGTCACCGTCATTGGTTAGT
ATCCCTGTCCACAACATGTGTCATGTCTCTGAACTCAAGACTAACCTCGCAACACAGACGCTCCCGGTCACCGTGATTT
CATCAAGAACATGA

(4) *Pestalotiopsis* sp. 3 (sp. code: Pe2)

Pe2-a_PB1-P2-T3-8
>ITS: Accession no. KP122294
>Tef1 (Pe2a_PB1-P2-T3-8)
AGAAGGTTAGTCATTTATTGATTCCCATCATCATCCCCCTTGACTTCAGCATCATAATTTTCAACCTACGTGTTGAAAAT
TATTTTCGCTCCTTCCACACTTTTTTCGCTGGTTACCCCGCCGCGAGGCACCCGCACGACCCCGCGGTGCAAACGAAAA
ATTTCTTATCACAGCCCCACCTTGCACAAGCAACCATGCATTGCTCATGAGACCCACTTTGAACAATTGCTAATGCCTTC
ATACAGGAAGCCGCCGAGCTCGGTAAGGGTTCCTTCAAGTACGCCTGGGTTCTTGACAAGCTCAAGGCCGAGCGTGAG
CGTGGTATCACCATCGATATCGCTCTCTGGAAGTTCGAGACCAACGAGTACAATGTCACCGTCATTGGTTAGTACCCCT

CCGCCTATGCCATGTGCTGCTCCATAAGACACTTGACTAACCTTGCTTCATAGACGCTCCCGGTCACCGTGATTTCATCA
AGAACATG

Pe2-b_SH2-P2-T1-2
>ITS: Accession no. KP122295
>Tef1 (Pe2b_SH2-P2-T1-2)
AGAAGGTTAGTCATCTACTGATTCCCGTCATCATTCTCCTTCACTTCAGCGTCATGATTTTCAGCCTACGTGTTGAAAAT
TATTTTCGCTCCTTCCACACTTTTTTCGCTGGTTACCCCGCCGCGAGGCACCAGCACGACCCCGCGGTGCAAACGAAAA
ATTTCTTATCACAGCCCCACCTTGCATAAGCAACCATGCATTGCTCATGAGATCCACTTTGAACAATTGCTAATGCCTTC
ATACAGGAAGCCGCCGAGCTCGGTAAGGGTTCCTTCAAGTACGCCTGGGTTCTTGACAAGCTCAAGGCCGAGCGTGAG
CGTGGTATCACCATCGATATCGCTCTCTGGAAGTTCGAGACCAACGAGTACAATGTCACCGTCATTGGTTAGTACCACT
CCACCTATGCCATGTGCTGCTCCATAAGACACTTGACTAACCTTGCTTCATAGACGCTCCCGGTCACCGTGATTTCATCA
AGAACATG

(5) *Phomopsis* sp. 1, (sp. code: Pho1)

Pho1-b, or PB2-P6-N2-9
>ITS: Accession no. KP122277
>Tef1(Pho1-b_PB2-P6-N2-9)
GAGAAGGAAGGTTAGTACAAATCACAATAACAGCACATGCTATCCTGCCCTTCAGTTGCACCTCGAACCACTGGCTCG
CGGCGGCCTGCTGTGGCTGCTCCGTCACACACTGGGGCGCATTTTCACCCCTCGTTCTGGATTTTCCATTTTCAGTGCGG
GTGCGGGGTGCGCGCTTATCAGGGAGCTTATCTCCACTCCCAAAACCCTGCTGCACCACTCCCTGTAACCACCACCACC
ATCAACAATCCTACTGCCCTTCAATACCACATGAAGAAAAGAATTCGCCAGAAACGATGCTAACAATTCATCCATACA
GCCGCCGAGCTTGGCAAGGGTTCCTTCAAGTACGCCTGGGTTCTGGACAAGCTGAAGGCCGAGCGTGAGCGTGGTATC
ACTATCGACATTGCCCTCTGGAAGTTCGAGACTCCCAAGTACTATGTCACCGTCATTGGTATGTTCCACAACAAAGCCT
CGCACAGCCTGCGGGCGGCATTTCCACACCAGTGGACCGAGTCGCCGAGTATGTTTACAATTTGGACGACACTCGCTAA
TGCCGTTCGCTTCCAGACGCTCCCGGTCACCGTGATTTCATCAAGAACAT

Pho1-d, or SH2-P4-N-Lf-2
>ITS: Accession no. KP122278
>Tef1(Pho1d_SH2-P4-N-Lf-2)
GAGAAGGAAGGTTAGTACAAATCACAATAACAGCACATGCTATCCTGCCCTTCAGTTGCACCTCGAACCACTGGCTCG
CGGCGGCCTGCTGTGGCTGCTCCGTCATACACTGGGGCGCATTTTCACCCCTCGTTCTGGATTTTCCATTTTCAGTGCGG
GTGCGGGGTGCGCGCTTATCAGGGAGCTTATCTCCACTCCCAAAACCCTGCTGCACCACTCCCTGTAACCACCACCACC
ATCAACAATCCTACTGCCCTTCAATACCACATGAAGAAAAGAATTCGCCAGAAACGATGCTAACAATTCATCCATACA
GCCGCCGAGCTTGGCAAGGGTTCCTTCAAGTACGCCTGGGTTCTGGACAAGCTGAAGGCCGAGCGTGAGCGTGGTATC
ACTATCGACATTGCCCTCTGGAAGTTCGAGACTCCCAAGTACTATGTCACCGTCATTGGTATGTTCCACAACAAAGCCT
CGCACAGCCTGCGGGCGGCATTTCCACACCAGTGGACCGAGTCGCCGAGTATGTTTACAATTTGGACGACACTCGCTAA
TGCCGTTCGCTTCCAGACGCTCCCGGTCACCGTGATTTCATCAAGAACAT

Pho1-e, or RU1-P6-N2-2
>ITS: Accession no. KP122277
>Tef1(Pho1e_RU1-P6-N2-2)
AGAAGGAAGGTTAGTACAAATCACAATAACAGCACATGCTATCCTGCCCTTCAGTTGCACCTCGAACCACTGGCTCGC
GGCGGCCTGCTGTGGCTGCTCCGTCATACACTGGGGCGCATTTTCACCCCTCGTTCTGGATTTTCCATTTTCAGTGCGG
TGCGGGGTGCGCGCTTATCAGGGAGCTTATCTCCACTCCCAAAACCCTGCTGCACCACTCCCTGTAACCACCACCACCA
TCAACAATCCTACTGCCCTTCAATACCACATGAAGAAAAGAATTCGCCAGAAAAGATGCTAACAATTCATCCATACAG
CCGCCGAGCTTGGCAAGGGTTCCTTCAAGTACGCCTGGGTTCTGGACAAGCTGAAGGCCGAGCGTGAGCGTGGTATCA
CTATCGACATTGCCCTCTGGAAGTTCGAGACTCCCAAGTACTATGTCACCGTCATTGGTATGTTCCACAACAAAGCCTC
GCACAGCCTGCGGGCGGCATTTCCACACCAGTGGACCGAGTCGCCGAGTATGTTTACAATTTGGACGACACTCGCTAAT
GCCGTTCGCTTCCAGACGCTCCCGGTCACCGTGNTTTCATCAAGAACATG

(6) *Phomopsis* sp. 2 (sp. code: Pho2)

Pho2-a, or RU2-P1-T-Lf-1
>ITS: Accession no. KP122280
>Tef1(Pho2_RU2-P1-T-Lf-1)

AGAAGGAAGGTTAGTAAATATCACAGTCACGGAACATGCTACCTGGCCCTCCATACTGCACCTCAATCATCAGCCCGC
AGATGCTCGCGCGGCCTCGCCATGTCGGGGGGCGCATTTTCACCCCTCGCTTTGGATTTTCCATTTTCAGTGCGAGTGCG
GGGTGCGCTTATCAGGGGGCGGGCTTATCTCCTACAACCAAAACCCTGTCACATCACTCACCCAATCCTTGTCACCACC
ACCAATACGCTCACCATCAACCCCATCGCCTCTTTCAATACAACTCGTGAAACGCGTCGAATATTATGCTGACCCTCTA
TCTACACAGCCGCCGAGCTTGGCAAGGGTTCCTTCAAGTACGCCTGGGTTCTGGACAAGCTGAAGGCCGAGCGTGAGC
GTGGTATCACTATCGACATTGCCCTCTGGAAGTTCGAGACTCCCAAGTACTATGTCACCGTCATTGGTATGTTTCCATCC
TTTATTGAACGTCCTTCGAGCTGCCGGTCCACACCAGAGGACCGAGTCGCTGTGGATGTTTCCAATCATGATAACACTC
GCTGACACGGTTACCTTCCAGACGCTCCCGGTCACCGTGACTTCATCAAGAACATG

(7) *Colletotrichum boninense sensu lato* sp. 1 (sp. code: Co1), winter/spring isolates:

Co1-b, or HW2-L-1

>GADPH (Co1-b_HW2-L-1)

GTCAACGACCCCTTCATTGAGACCAAGTACGCCGTGAGTGTCACCCCACTTTACCCCTCTAAGTTCATCCGTGATATTAC
GCACGCCCGCCCACCCCCCAGCTTCCTCCTGCCGCCAGCGCTGGGCGTTCGGAAAGGCAGCAGCCATTCAATCTAAGCT
CTCAATCGAAAGCAATTGATGGAACTGGTTGGAACAGCGGGGTGTGATAAATTAGATAGATTTTCCACCCAAGCTCCC
AAAAACGCTCGCTGACTTGCCTTTCCTAGGCCTACATGCTCAAGTACGACTCC

Co1-c, or CP5-H2

>GADPH (Co1-c_CP5-H2)

GTCAACGACCCCTTCATTGAGACCAAGTACGCCGTGAGTGTCACCCCACTTTACCCCTCTAAGTTCATCGTGATATTAC
GCACGCCCGCCCACCCCCCAGCTTCCTCCTGCCGCCAGCGCTGGGCGTTCGGAAAGGCAGCAGCCATTCAATCTAAGCT
CTCAATCGAAAGCAATTGATGGAACTGGTTGGAACAGCGGGGTGTGATAAATTAGATAGATTTTCCACCCAAGCTCCC
AAAAACGCTCGCTGACTTGCCTTTCCTAGGCCTACATGCTCAAGTACGACTCC

Co1-d, or NA4-EE

>GADPH (Co1-d_NA4-EE)

CGTCAACGACCCCTTCATTGAGACCAAGTACGCCGTGAGTGTCACCCCACTTTACCCCTCTAAGTTCATCGTGATATTAC
GCACGCCCGCCCACCCCCCAGCTTCCTCCTGCCGCCAGCGCTGGGCGTTCGGAAAGGCAGCAGCCATTCAATCTAAGCT
CTCAATCGAAAGCAATTGATGGAACTGGTTGGAACAGCGGGGTGTGATAAATTAGATAGATTTTCCACCCAAGCTCCC
AAAAACGCTCGCTGACTTGCCTTTCCTAGGCCTACATGCTCAAGTACGACTCC

(8) *Colletotrichum boninense sensu lato* sp., summer isolate (sp. code: Co1) probably different sp. from winter in retrospect, but retains the same code):

Co1-a, or RU2-P3-N1-7

>ITS: Accession no. KP122274

>GAPDH (Co1-a_RU2-P3-N1-7)

ACCCCTTCATTGACACCAANTACGCTGTGAGTATCACCCCACTTACCCCTCCAAGGTCGTCATGATATCAAGCCCGCCA
ACCCGGCCAACCGCCCTTCGCCGCAGGAGCCTGGCAGCCAACGGACACGAGTTCCCAGACACCGCCAGTGGCCGAGAT
AGTGGGATGTGATACTCGTTTGGCTCAACAAAGCTTCCAAGCCACTCGCTGACTCGCCCTCCGCAGGCCTACATGCTCA
AGTAC

(9) *Colletotrichum acutatum sensu lato* sp., summer isolates, (sp. code: R1)

R1-a, or SH1-P6-N3-2

>GAPDH_(R1-a_SH1-P6-N3-2)

CCCCTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCACTTTACCCCTCCATGATGATATCACGTCTGCTACAATAA
CACCAGCTTCATCGGTAACCACGGGAAAAGAGCCAGAGCTAGTACTCTCGACTCTTTGGACCCAAGGTTTCGATTGGGC
TCGTTGTTGTAATGATACGACGTGACACAATCATGCAGAAACAGCCCAAACAAAATTTGCTGACAGACAATCATCACA
GGCCTACATGCTCAAGTAC

R1-b, or RU3-P2-N3-8

>ITS: Accession no. KP122275

>GAPDH (R1-b_RU3-P2-N3-8)

GCCGTCAACGACCCCTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCACTTTACCCCTCCATAATGATATCACGT
CTGCTACAATAACACCAGCTTCATCGGTAACCACGGGAAAAGAGTCAGAGCTAGTACTCTCGACTCTTTGGACCCAAG
GTTTCGATTGGGCTCGTTGTTGTAATGATACGACGTGACACAATCATGCAGAAACAGCCCAAACAAAATTTGCTGACAG
ACAATCATCACAGGCCTACATGCTCAAGTACG

R1-e_SH2-P2-N-Lf-3
>ITS: Accession no. KP122276
>GAPDH (R1-e_SH2-P2-N-Lf-3)
CTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCACTTTACCCCTCCATAATGATATCACGTCTGCTACAATAACA
CCAGCTTCATCGGTAACCACGGGAAAAGAGTCAGAGCTAGTACTCTCGACTCTTTGGACCCAAGGTTTCGATTGGGCTC
GTTGTTGTAATGATACGACGTGACACAATCATGCAGAAACAGCCCAAACAAAATTTGCTGACAGACAATCATCACAGG
CCTACATGCTCAAGTAC

(10) *Phyllosticta* sp. 1 (sp. code: Ph1)

Isolate: Ph1-b, or PB1-P3-N-Lf-3
>ITS: Accession no. KP122281
>Tef1(Ph1b_PB1-P3-N-Lf-3)
GGTTCGCTCCTTGCGCCNCCTTTTGCCGTCTCGCTCGAGGGGCTGCCTTTTTTTTTTTTTTTGGNGGGGTCGGGCTGCGCT
AGGCCGCTTTGGCGCGTCTCGGCAACATCGCCCGAAGCAGCATTTTCGCGCCCGACCCCGTCGCCTTGTGCACTCGCTT
CACTTTGGTCGCAACATTTTTGCTAACGCCCGCACAGGAAGCCGCCGAGCTCGGCAAGGGTTCCTTCAAGTACGCCTGG
GTCCTCGACAAGCTGAAGGCTGAGCGTGAGCGTGGTATCACCATCGACATTGCTCTCTGGAAGTTCGAGACCCCGAAGT
ACTA

Isolate: Ph1-f, or RU1-P2-N2-7
>ITS: Accession no. KP122282
>Tef1(Ph1f_RU1-P2-N2-7)
CGGTTCGCTCCTTGCGCCNCCTTTTGCCGTCTCGCTCGAGGGGCTGCCTTTTTTTTTTTTTTGGNGGGGTCGGGCTGCGCTA
GGCCGCTTTGGCGCGTCTCGGCAACATCGCCCGAAGCAGCATTTTCGCGCCCGACCCCGTCGCCTTGTGCACTCGCTTC
ACTTTGGTCGCAACATTTTTGCTAACGCCCGCACAGGAAGCCGCCGAGCTCGGCAAGGGTTCCTTCAAGTACGCCTGGG
TCCTCGACAAGCTGAAGGCTGAGCGTGAGCGTGGTATCACCATCGACATTGCTCTCTGGAAGTTCGAGACCCCGAAGTA
CTA

Isolate: Ph1-d, or SH2-P5-N-Lf-3
>Tef1(Ph1d_SH2-P5-N-Lf-3)
TCGAGAAGGTCAGTCGCCTCACATTTTCTTTGAGCGCAGGGCGGCCAGTTCGTCGCGCCACCTTTTGCCGTCTCGCTCCA
TGGCCATTTTTTGGTGGGGTCGGGCTGCGCTAGGCTGCTTTGGCGCATTCGGCAATATCGCCCGAAGCAGCATTTTTGC
GCCCGACGGTCGCCCTGCGCACTCACTTCACATCAATCGCAACATTCTGCTAACGCCCTCGTAGGAAGCCGCCGAGCTC
GGCAAGGGTTCCTTCAAGTACGCCTGGGTCCTCGACAAGCTGAAGGCTGAGCGTGAGCGTGGTATCACCATCGACATT
GCTCTCTGGAAGTTCGAGACCCCGAAGTACTATGTCACCGTCATTGACGCCCCCGGTCACCGTGACTTCATCAAGAACA
TGA

(11) *Phyllosticta* sp. 2 (sp. code: Ph3)

Ph3-a, or PB1-P5-N3-6
> ITS: Accession no. KP122283
> Tef1(Ph3-a_PB1-P5-N3-6)
AAGGTCAGTCGCCTCACATTTTCTTTGAGCGCAGGGCGGCCAGTTCGTCGCGCCACCTTTTGCCGTCTCGCTCCATGGCC
ATTTTTTGGTGGGGTCGGGCTGCGCTAGGCTGCTTTGGCGCATTCGGCAATATCGCCCGAAGCAGCATTTTTGCGCCCG
ACGGTCGCCCTGCGCACTCACTTCACATCAATCGCAACATTCTGCTAACGCCCTCGTAGGAAGCCGCCGAGCTCGGCAA
GGGTTCCTTCAAGTACGCCTGGGTCCTCGACAAGCTGAAGGCTGAGCGTGAGCGTGGTATCACCATCGACATTGCTCTC
TGGAAGTTCGAGACCCCGAAGTACTATGTCACCGTCATTGACGCCCCCGGTCACCGTGACTTCATCAAGAACATG

Ph3-d, or RU1-P2-N-Lf-1
>ITS: Accession no. KP122284

Ph3-e, or SH1-P2-N2-6
>ITS: Accession no. KP122285
>Tef1(Ph3e_SH1-P2-N2-6)
AGAAGGTCAGTCGCCTCACATTTTCTTTGAGCGCAGGGCGGCCAGTTCGTCGCGCCACCTTTTGCCGTCTCGCTCCATG
GCCATTTTTTGGTGGGGTCGGGCTGCGCTAGGCTGCTTTGGCGCATTCGGCAATATCGCCCGAAGCAGCATTTTTGCGC
CCGACGGTCGCCCTGCGCACTCACTTCACATCAATCGCAACATTCTGCTAACGCCCTCGTAGGAAGCCGCCGAGCTCGG
CAAGGGTTCCTTCAAGTACGCCTGGGTCCTCGACAAGCTGAAGGCTGAGCGTGAGCGTGGTATCACCATCGACATTGCT
CTCTGGAAGTTCGAGACCCCGAAGTACTATGTCACCGTCATTGACGCCCCCGGTCACCGTGACTTCATCAAGAACATGA

(12) Mycelia sterilia A-Tan (sp. code: My1, or My1b)

My1, or 50A-N-1t-12
>ITS: Accession no. KP122256.1

My1-a, or PB1-P5-N2-2
>ITS: Accession no. KP122268.1

My1-e, or RU3-P2-N3-4
>ITS: Accession no. KP122269.1 (570 bp)
>LSU: Accesssion no. KP137621 (1,310 bp)

(13) Mycelia sterilia A-Yellow (sp. code: MyY, or My1y)

My1y-c, or SH2-P2-N1-2
>ITS: Accession no. KP122270.1 (666 bp)
>LSU: Accesssion no. KP137622.1 (769 bp)

My1y-d, or RU1-P5-N2-3
>ITS: Accession no. KP122271.1 (666 bp)
>LSU: Accession no. KP137623 (797 bp)

(14) Mycelia sterilia B-White (*Anthostomella* sp.) (sp. code: W2)

W2-a, or PB1-P3-N2-4
>ITS: Accession no. KP122263.1

W2-b, or SH2-P5-T2-7
>ITS: Accession no. KP122264.1

(15) Mycelia sterilia E-Pink (sp. code: W2p)

W2p-a, or PB1-P4-N-lf-1
>ITS: Accession no. KP122272.1

W2p-b, or SH2-P4-T-lf-1
>Accession no. KP122273.1

(16) Mycelia sterilia C-White (sp. code: W3)

W3-d, or RU3-P3-N1-1
>Accession no. KP122265.1

(17) *Diplodia* sp. (*Botryosphaeria* sp. 2) (sp. code: G2)

G2, or 60-A-T-5b-7
>ITS: Accession no. KP122262.1 (554 bp)

(18) *Coniothyrium*-like sp. 1A (sp. code: M1B)

M1B, or 60A-T-1b-7
>ITS: Accession no. KP122258

(19) *Coniothyrium*-like sp. 1B (sp. code: M1D)

M1D, or 50A-T-3b-1
>ITS: Accession no. KP122259

(20) *Phoma*-like sp. 2 (sp. code: M1C)

M1C-a, or RU2-P1-T3-3
>ITS: Accession no. KP122266

M1C-b, or SH1-P4-T2-6
>ITS: Accession no. KP122267

(21) Coniothyrium-like sp. 2A (sp. code M4)

M4, or 160A-T-1t-4
>ITS Accession no. KP122260

(22) *Coniothyrium*-like sp. 3 (sp. code: M5)

M5, or 160B-T-5t-7
>ITS: Accession no. KP122261

(23) *Phoma*-like sp. 1 (sp. code:M7)

M7_60B-T-3t-3
>ITS: Accession no. KP122257

(24) *Sporobolomyces* sp. (red yeast sp.) (sp. code: RY)

RY, or 10B-T-2t-1
>ITS + much LSU (1,388 bp total): Accession no. KP122300

(25) ***Alternaria*** sp. (sp. code: originally M1CAl, or switched in some tables to 'Alt.')

M1CAl-a, or RU1-P5-T3-5
>ITS: Accession no. KP122286

M1CAl-c, or PB2-P4-T1-6
>ITS: Accession no. KP122287

Appendix F: Measurements of conidia

Format: Average ± standard deviation (minimum - maximum)

(1) *Pestalotiopsis* sp. 1 (Ch. 3 & 4), species code Pe4

Isolate: 'Pe4d'
Medium: autoclaved greenbrier stem inoculated with isolate in wet chamber petri dish.
Sample Size: n=53; except for apical appendage, n = 105
Basal cell length: 4.7 ± 0.7 (3.0-6.9)
1st middle cell length x width = 5.9 ± 0.8 (3.5 - 8.2) x 6.1 ± 0.7 (4.4 - 7.6)
2nd middle cell length x width = 5.9 ± 0.9 (4.1-8.6) x 7.2 ± 0.6 (6.0 - 8.4)
3rd middle cell length x width = 6.1 ± 0.6 (4.7 - 7.7) x 6.7 ± 0.5 (5.2-7.6)
Apical cell length = 4.9 ± 0.8 (3.5 - 7.7)
Apical appendage = 20.1 ± 3.8 (10.6 - 30.2)
Total length = 27.7 ± 2.5 (22.8 - 33.0)

'Pe4a'
Medium: same as above
Sample Size: n=57 for most; total length n=56; apical appendage n=113)
Basal cell length = 5.3 ± 1.0 (3.4 - 8.7)
1st middle cell length x width = 6.9 ± 0.8 (5.5 - 8.4) x 5.9 ± 0.6 (4.5 - 7.0)
2nd middle cell length x width = 6.8 ± 0.6 (5.8 - 8.8) x 7.5 ± 0.5 (6.5 - 8.7)
3rd middle cell length x width = 6.8 ± 0.8 (4.5 - 8.1) x 6.9 ± 0.5 (5.9 - 7.9)
Apical cell length = 4.9 ± 0.7 (3.0 - 6.6)
Apical appendage = 25.4 ± 4.4 (13.8 - 33.9)
Total length = 30.7 ± 2.0 (25.9 - 36.1)

Pe4c, autoclaved stem
n=51 for most; apical appendage n=102)
Basal cell length: 6.3 ± 1.0 (4.7 - 8.9)
1st middle cell length x width = 8.1 ± 1.0 (5.7 - 10.1) x 5.5 ± 0.6 (4.1 - 7.2)
2nd middle cell length x width = 7.8 ± 0.7 (6.5 - 9.7) x 7.1 ± 0.7 (5.6 - 8.9)
3rd middle cell length x width = 7.9 ± 0.7 (6.6 - 9.8) x 6.6 ± 0.6 (5.4 - 8.0)
Apical cell length = 5.5 ± 0.8 (3.2 -7.4)
Apical appendage = 22.7 ± 4.0 (13.9 - 33.9)
Total length = 35.3 ± 2.4 (29.7 - 41.2)

(2) *Pestalotiopsis* sp. 2A (Ch. 3 & 4), species code Pe1L

Pe1La, autoclaved stems
n=53; apical appendages n=106
Basal cell length: 5.0 ± 0.7 (2.6 - 6.3)
1st middle cell length x width = 5.5 ± 0.7 (4.0 - 6.6) x 5.3 ± 0.6 (4.0 - 6.6)
2nd middle cell length x width = 5.3 ± 0.6 (4.1 - 6.6) x 6.9 ± 0.7 (5.7 - 8.6)
3rd middle cell length x width = 5.2 ± 0.6 (4.0 - 6.4) x 6.2 ± 0.6 (5.2 - 7.5)

[3-central cells length = 16.0 ± 1.4 (12.8 - 18.7)]
Apical cell length = 4.1 ± 0.6 (2.8 - 6.1)
Apical appendage = **15.1** ± 3.0
Total length = 25.0 ± 1.8

Pe1Lb, autoclaved stems
n=55; apical appendages n=105
Basal cell length: 5.4 ± 0.7 (3.6 - 6.7)
1st middle cell length x width = 5.8 ± 0.7 (4.7 - 7.5) x 6.2 ± 0.9 (4.1 - 7.8)
2nd middle cell length x width = 5.6 ± 0.7 (4.2 - 7.3) x 8.2 ± 1.4 (5.2 - 10.9)
3rd middle cell length x width = 5.6 ± 0.7 (4.1 - 6.8) x 7.1 ± 1.1 (5.0 - 9.8)
[3-central cells length = 17.0 ± 1.5 (14.0 - 20.5)]
Apical cell length = 4.1 ± 0.8 (2.6 - 6.0)
Apical appendage = 16.3 ± 3.3
Total length = 26.2 ± 2.0 (22.2 - 30.2)

Pe1Lc, autoclaved stems
n=30; apical appendages n=42
Basal cell length: 5.0 ± 1.0 (3.2 - 7.2)
1st middle cell length x width = 5.4 ± 0.7 (4.1 - 6.5) x 5.4 ± 0.5 (4.2 - 6.5)
2nd middle cell length x width = 5.6 ± 0.6 (4.5 - 7.2) x 6.7 ± 0.5 (5.6 - 7.6)
3rd middle cell length x width = 4.9 ± 0.6 (3.9 - 6.1) x 5.8 ± 0.5 (5.1 - 6.8)
Apical cell length = 4.9 ± 0.9 (3.5 - 7.0)
[3-central cells length = 15.9 ± 1.1 (13.8 - 18.0)]
Total length (all 5 cells) = 26.0 ± 1.8 (22.4 - 29.6)
Apical appendage = **25.9** ± 7.9 (11.9 - 42.4)

(3) *Pestalotiopsis* sp. 2B (Ch. 3 & 4), species code Pe1D

Pe1Da, autoclaved stems
n=31; apical appendages n=59
Basal cell length: 4.1 ± 0.7 (2.8 - 5.7)
1st middle cell length x width = 5.0 ± 0.6 (4.0 - 6.5) x 5.9 ± 07 (4.5 - 8.1)
2nd middle cell length x width = 4.9 ± 0.7 (3.3 - 6.2) x 7.1 ± 0.8 (5.5 - 9.1)
3rd middle cell length x width = 4.8 ± 0.5 (3.5 - 5.7) x 6.3 ± 0.6 (5.0 - 8.0)
Apical cell length = 3.5 ± 0.6 (2.6 - 4.8)
[3-central cells length = 14.6 ± 1.0 (12.8 - 16.6)]
Total length (all 5 cells) = 21.8 ± 2.8 (18.9 - 25.0)
Apical appendage = 14.2 ± 2.8 (8.9 - 20.4)

Pe1Db, autoclaved stems
n=64; apical appendages n=122
Basal cell length: 4.9 ± 0.8 (2.8 - 7.2)
1st middle cell length x width = 5.5 ± 0.6 (4.3 - 7.1) x 5.9 ± 0.8 (4.1 - 7.8)
2nd middle cell length x width = 5.3 ± 0.6 (4.3 - 6.6) x 7.3 ± 1.0 (4.7 - 10.2)
3rd middle cell length x width = 5.0 ± 0.7 (3.1 - 6.8) x 6.3 ± 0.8 (4.7 - 8.8)

Apical cell length = 4.0 ± 0.7 (2.8 - 6.6)
[3-central cells length = 15.8 ± 1.3 (13.2 - 19.2)]
Total length (all 5 cells) = 24.3 ± 1.7 (20.4 - 28.1)
Apical appendage = 14.4 ± 3.1 (7.1 - 22.2)

Pe1Dc, autoclaved stems
n=35; apical appendages n=70
Basal cell length: 5.2 ± 0.8 (3.9 - 7.2)
1st middle cell length x width = 5.6 ± 0.6 (3.8 - 7.1) x 5.7 ± 0.9 (3.6 - 8.3)
2nd middle cell length x width = 5.4 ± 0.9 (3.4 - 7.3) x 7.0 ± 1.3 (5.3 - 10.6)
3rd middle cell length x width = 5.2 ± 0.7 (3.8 - 6.7) x 6.1 ± 1.1 (4.2 - 9.4)
Apical cell length = 3.8 ± 0.7 (2.5 - 5.3)
[3-central cells length = 16.2 ± 1.8 (11.2 - 20.0)]
Total length (all 5 cells) = 24.6 ± 2.1 (20.6 - 28.5)
Apical appendage = 12.7 ± 3.4 (1.5 - 20.2)

(4) *Pestalotiopsis* sp. 3 (Ch. 3 & 4), species code Pe2

Pe2a, autoclaved stems
n=40; apical appendages n=80
Basal cell length: 5.6 ± 1.5 (3.5 - 10.2)
1st middle cell length x width = 6.4 ± 1.6 (5.1 - 12.7) x 5.5 ± 1.3 (3.7 - 10.5)
2nd middle cell length x width = 6.0 ± 1.3 (4.4 - 10.5) x 7.5 ± 1.9 (5.8 - 15.5)
3rd middle cell length x width = 5.6 ± 1.6 (3.7 - 11.2) x 6.9 ± 1.7 (5.2 - 13.4)
Apical cell length = 4.6 ± 1.3 (3.1 - 8.9)
[3-central cells length = 18.0 ± 4.3 (13.3 - 33.0)]
Total length (all 5 cells) = 27.7 ± 6.8 (21.4 - 52.9)
Apical appendage = 25.1 4.2 ± 5.9 (16.9 - 46.2)

Pe2b, autoclaved stems
n=40; apical appendages n=40
Basal cell length: 5.2 ± 0.9 (3.5 - 7.6)
1st middle cell length x width = 5.9 ± 0.7 (3.8 - 7.5) x 5.6 ± 0.7 (3.6 - 7.5)
2nd middle cell length x width = 5.2 ± 0.7 (2.9 - 7.3) x 7.0 ± 0.6 (5.9 - 8.8)
3rd middle cell length x width = 5.2 ± 0.6 (3.8 - 6.7) x 6.2 ± 0.5 (5.3 - 7.5)
Apical cell length = 4.2 ± 0.7 (3.0 - 5.7)
[3-central cells length = 16.4 ± 1.4 (13.5 - 20.5)]
Total length (all 5 cells) = 25.4 ± 2.3 (20.9 - 30.3)
Apical appendage = 21.2 ± 3.8 (14.2 - 28.8)

(5) *Phomopsis* sp. 1 (Ch. 3 & 4), species code Pho1

Pho1E
Beta conidia length x width (n=30): 27.1 ± 3.3 (19.5 - 32.5) x 1.3 ± 0.3 (0.9 - 2.0)
Alpha conidia length x width (n=3): 10.3 ± 0.3 (10.1 - 10.6) x 3.8 ± 0.2 (3.7 - 4.1)
Alpha L/W ratio (n=3): 2.7 ± 0.1 (2.6 - 2.7)

Pho1B
Beta conidia length x width: 27.6 ± 3.5 (19.6 - 35.4) x 1.0 ± 0.2 (0.6 - 1.4)
Alpha conidia length x width(n=30): 10.7 ± 1.2 (8.7 - 13.1) x 3.8 ± 0.4 (2.7 - 4.7)
Alpha L/W ratio (n=30): 2.9 ± 0.6 (2.0 - 4.2)

Pho1D
Beta conidia length x width: 25.2 ± 3.6 (16.2 - 32.6) x 1.6 ± 0.4 (1.0 - 2.5)
Alpha conidia length x width: none produced

(7) *Colletotrichum boninense*, species code Co1, (winter isolate, different colony morphology from the rare summer isolates)

Co1, winter isolate (Feb. 2014), unknown isolate no.
Conidia length x width (n=30): 15.8 ± 1.2 (12.6 - 18.0) x 6.6 ± 0.5 (5.5 - 7.7)
Ratio L/W (n=30): 2.4 ± 0.3 (1.9 - 3.0)

Appendix G: Photos of fungal morphotypes: colonies and conidia

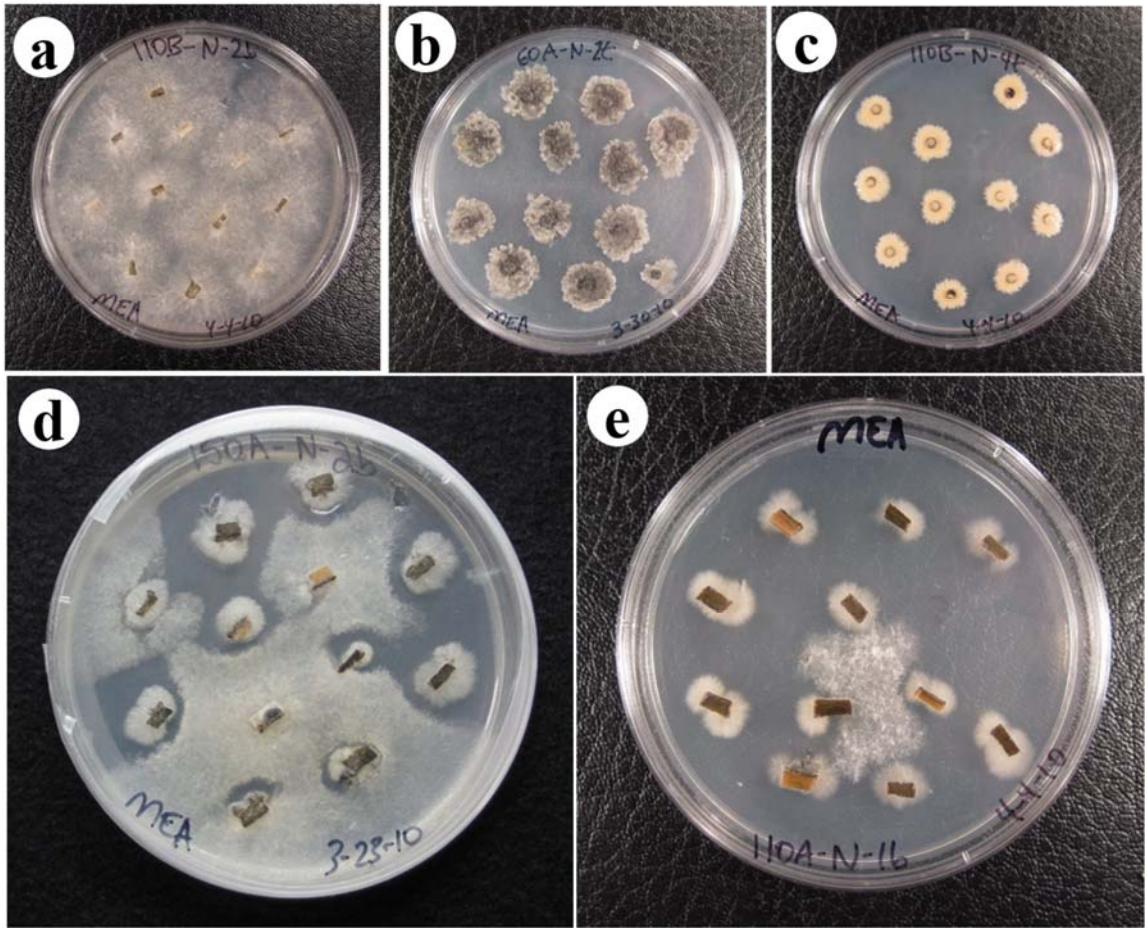

Fig. A-3. Endophytic colonies emerging from stems

Original surface sterilized stems plated on Difco MEA (100 mm Petri dishes) as described in Ch. III (the winter study). Twelve segments derived from a 1-cm sample should be present per plate unless one has been transferred to protect it from overgrowth, or if one was damaged or lost in the cutting process. **(a)** *Phomopsis* sp. 1 (sp. code: Pho1) is seen emerging from all segments, **(b)** *Phyllosticta* sp. 1 (sp. code: Ph1) is seen emerging from all segments. **(c)** *Aureobasidium pullulans* (sp. code: Au) is seen emerging from all segments. **(d)** Shows the interaction of *Colletotrichum boninense sensu lato* sp. (sp. code: Co1), the small dense and slow growing colonies, with *Phomopsis* sp. 1 (sp. code: Pho1), the fast growing expansive colonies. A zone of inhibition can be clearly seen slowing or preventing advance of Pho1 around the Co1 colonies. **(e)** Another Co1/Pho1 interaction plate. Co1 is seen emerging from all stem segments. Pho1 looks to be emerging from only one segment and its expansion appears to be arrested.

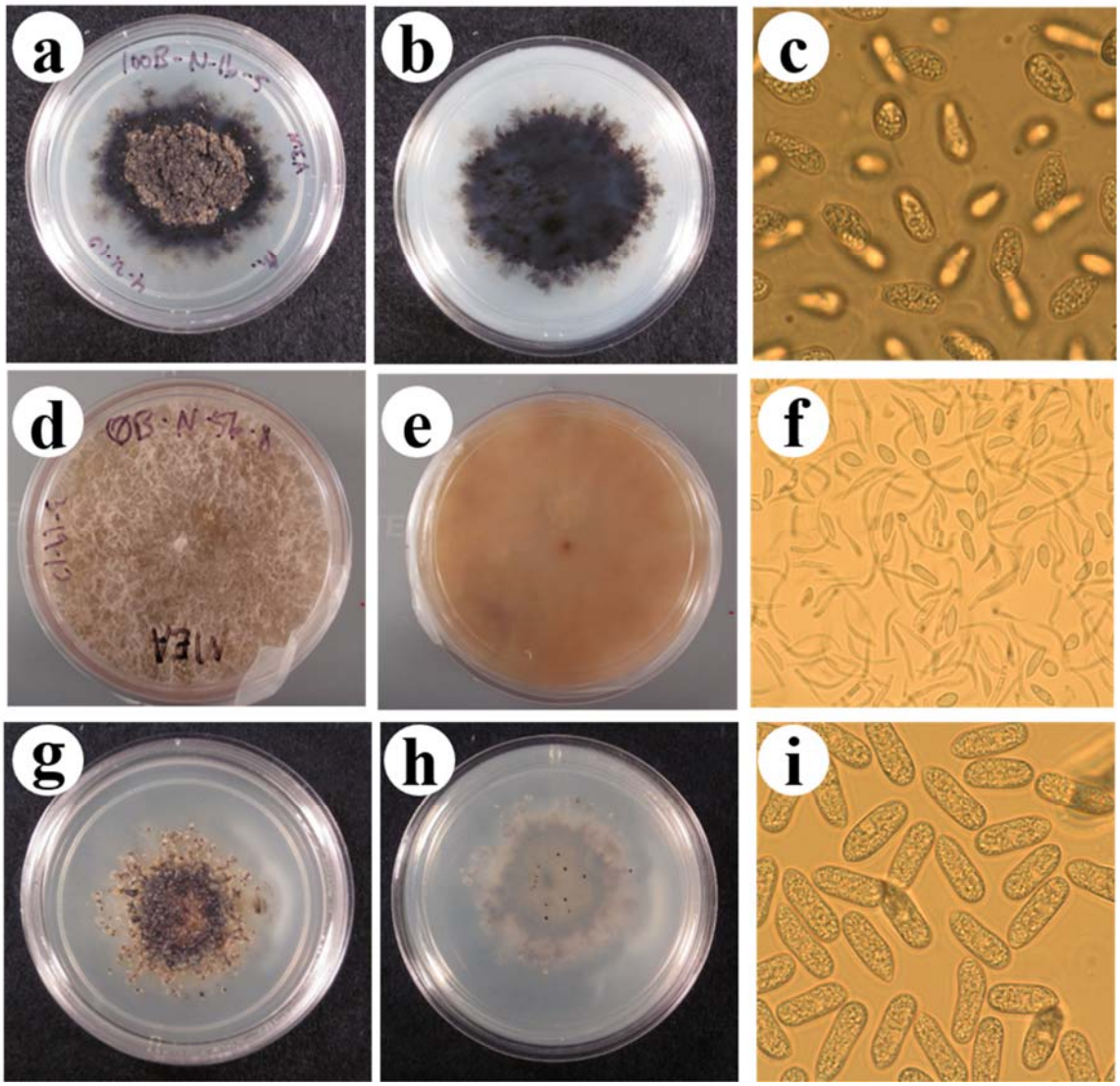

Fig. A-4. Dominant coelomycetous endophytes in winter

The three most commonly isolated endophytes of common greenbrier stems in the winter study (Ch. III). Colonies on Difco malt extract agar, from above, below, and conidia (produced on MEA or on autoclaved stem in wet chamber): **(a-c)** *Phyllostica* sp. 1 (sp. code: Ph1), 60 mm Petri dish, **(d-f)** *Phomopsis* sp. 1 (sp. code: Pho1), 100 mm Petri dish, **(g-i)** *Colletotrichum boninense sensu lato* sp. (sp. code: Co1), 60 mm Petri dish.

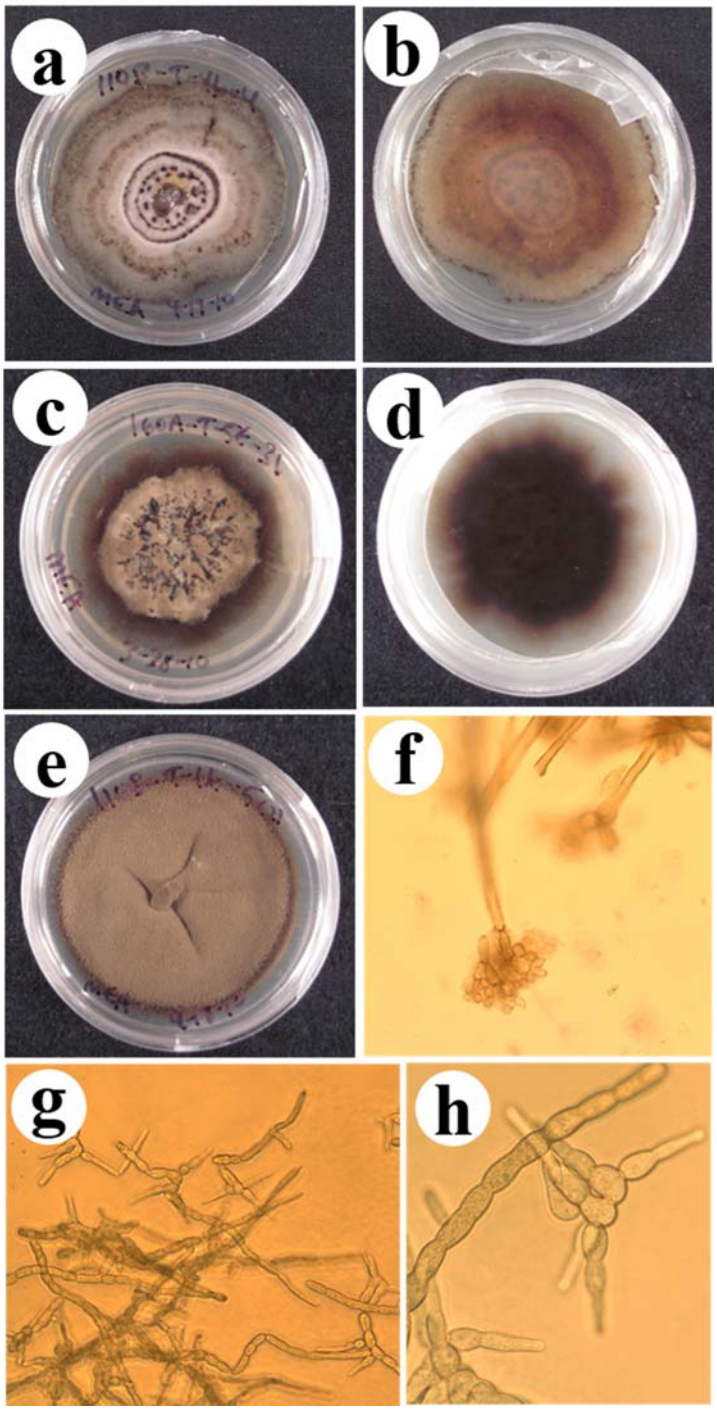

Fig. A-5. Common hyphomycetes of greenbrier stems in winter

Colonies on Difco MEA, 60 mm plates: **(a,b)** *Aureobasidium pullulans*, **(c,d)** *Cladosporium cladosporioides* (sp. code: Cld) colony with typical slightly deteriorated appearance, **(e)** *C. cladosporioides*, less commonly seen form of pristine colony **(f)** *C. cladosporioides* conidia and conidiophore **(g,h)** *Tripospermum myrti* (sp. code: Tr or TRP, winter and summer study respectively), lower and higher levels of magnification; image of colony unavailable, dark, slow growing, small colonies, tend to form peak; with better conidial production on water agar or PDA.

.

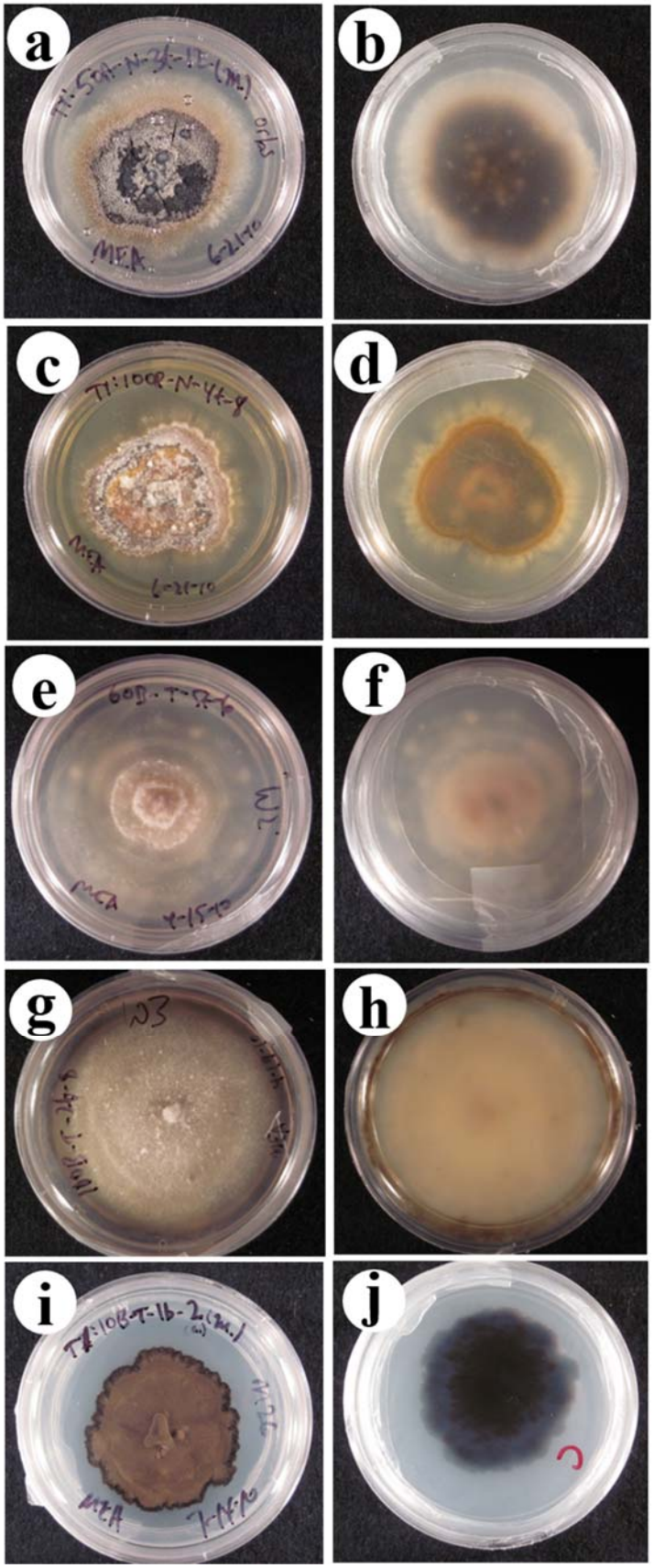

Fig. A-6. Common non-sporulating morphotypes of greenbrier

These isolates were all derived from the winter study, shown in 60 mm isolation plates, Difco MEA, top and bottom view of each. Several of these were more common in the summer study:

(a,b) Mycelia sterilia A-Tan (sp. code: My1, or My1b in summer study);

(c,d) Mycelia sterilia A-Yellow (sp. code MyY, or My1y in summer study);

(e,f) Mycelia sterilia B-White (*Anthostomella* sp.),' (sp. code: W2);

(g,h) Mycelia sterilia C-White (sp. code: W3);

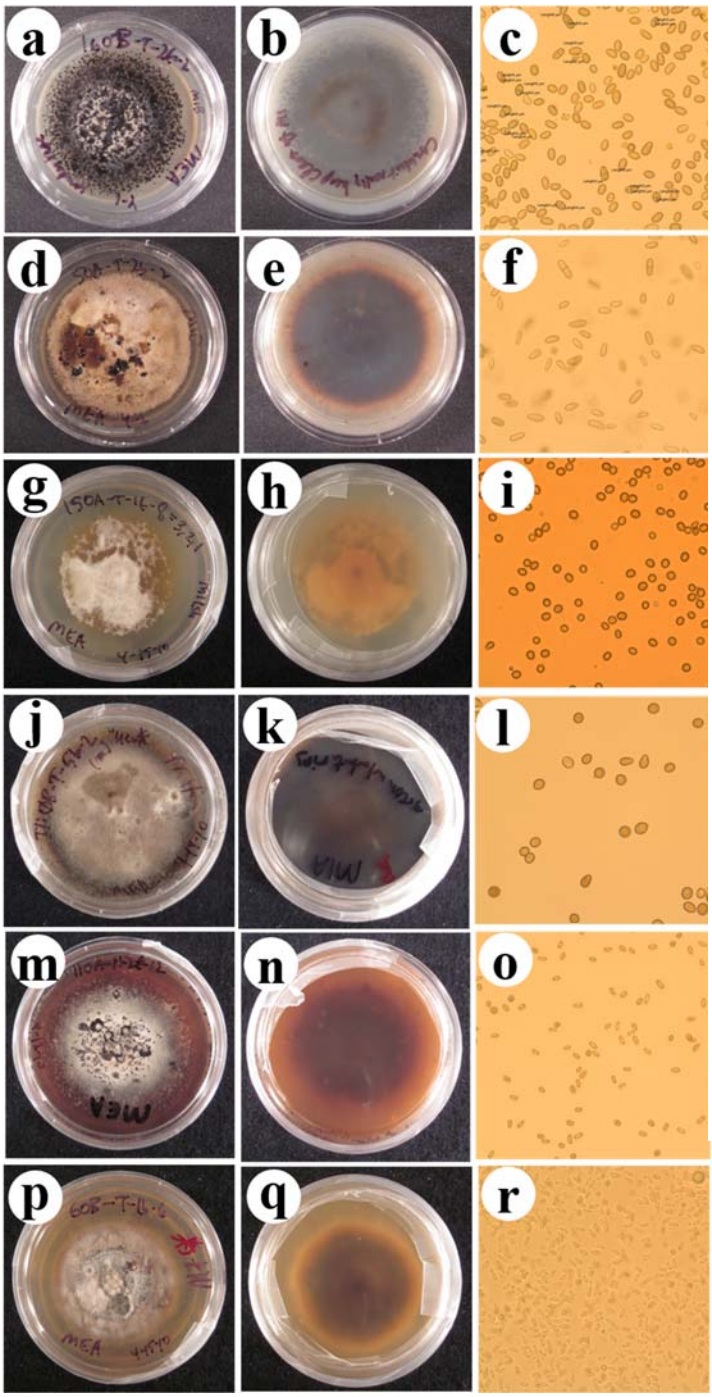

Fig. A-7. Common coelomycetes, predominantly epiphytic, seen in the winter study

(a-c) *Coniothyrium*-like sp. 1A (sp. code: M1B);

(d-f) *Coniothyrium*-like sp. 1B (sp. code: M1D);

(g-i) *Coniothyrium*-like sp. 2A (sp. code: M4);

(j-l) Coniothyrium-like sp. 2B (sp. code: M1A);

(m-o) *Coniothyrium*-like sp. 3 (sp. code: M5);

(p-r) *Phoma*-like sp. 1 (sp. code: M7);

ITS sequences suggest these isolates are in the class **Pleosporales,** except for *Coniothyrium*-like sp. 2B **(j-l),** which may also be, but was not sequenced. Some slightly variant colony morphologies were grouped in these categories based on conidial and pycnidial morphology, so these photos are not alone representative of the total morphotypes lumped in each category (e.g. *Coniothyrium*-like sp. 3 includes morphotypes without orange colored exudates).

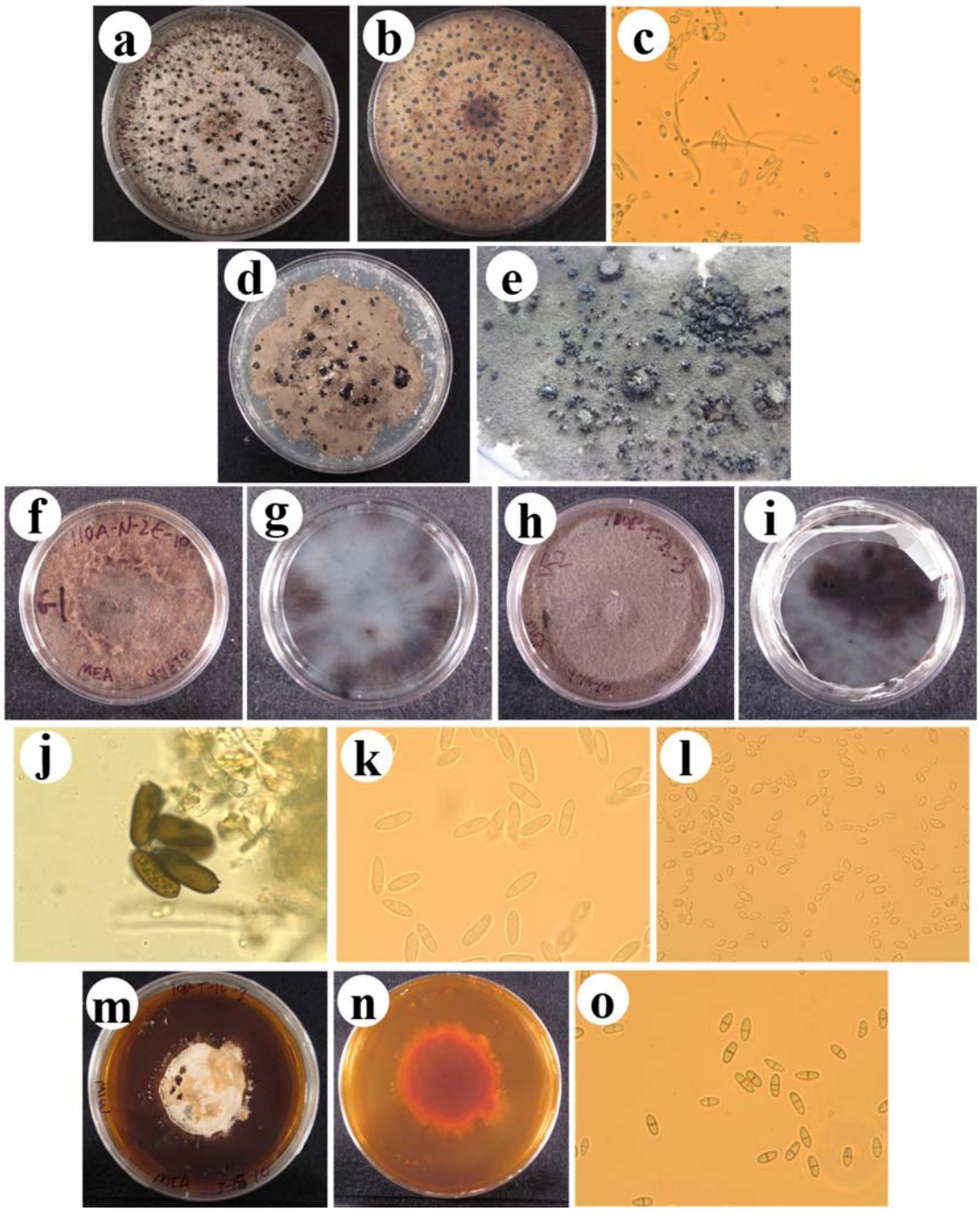

Fig. A-8. More common coelomycetes from the winter study

Some of these were also common in the summer study. Colonies all grown on Difco MEA: **(a,b,c)** *Phomopsis* sp. 2, (sp. code: Pho2) 100 mm plate, above, below, and conidia, **(d,e)** *Phyllosticta* sp. 2 (sp. code: Ph3), overhead view, 2 different isolates, 100 mm plates, **(f,g)** *Botryosphaeria* sp. 1 (sp. code: G1), 100 mm plate, above and below, **(h, i)** *Botryosphaeria* sp. 2 (a.k.a. *Diplodia* sp.) (sp. code: G2), 100 mm plate, above and below (also had very different growth rate from the previous, G1), **(j)** *Botryosphaeria* sp. 1 (speices code: G1), spores, **(k)** *Colletotrichum acutatum sensu lato* (species code: R1) conidia, **(l)** *Phoma*-like sp. 2 (sp. code: M1C) conidia, colony photo unavailable, dark colonies with pink fluid emerging from pycnidia, **(m,n,o)** *Ascochyta*-like sp. 1 (sp. code: M1W), 100 mm plates, above, below, and conidia.

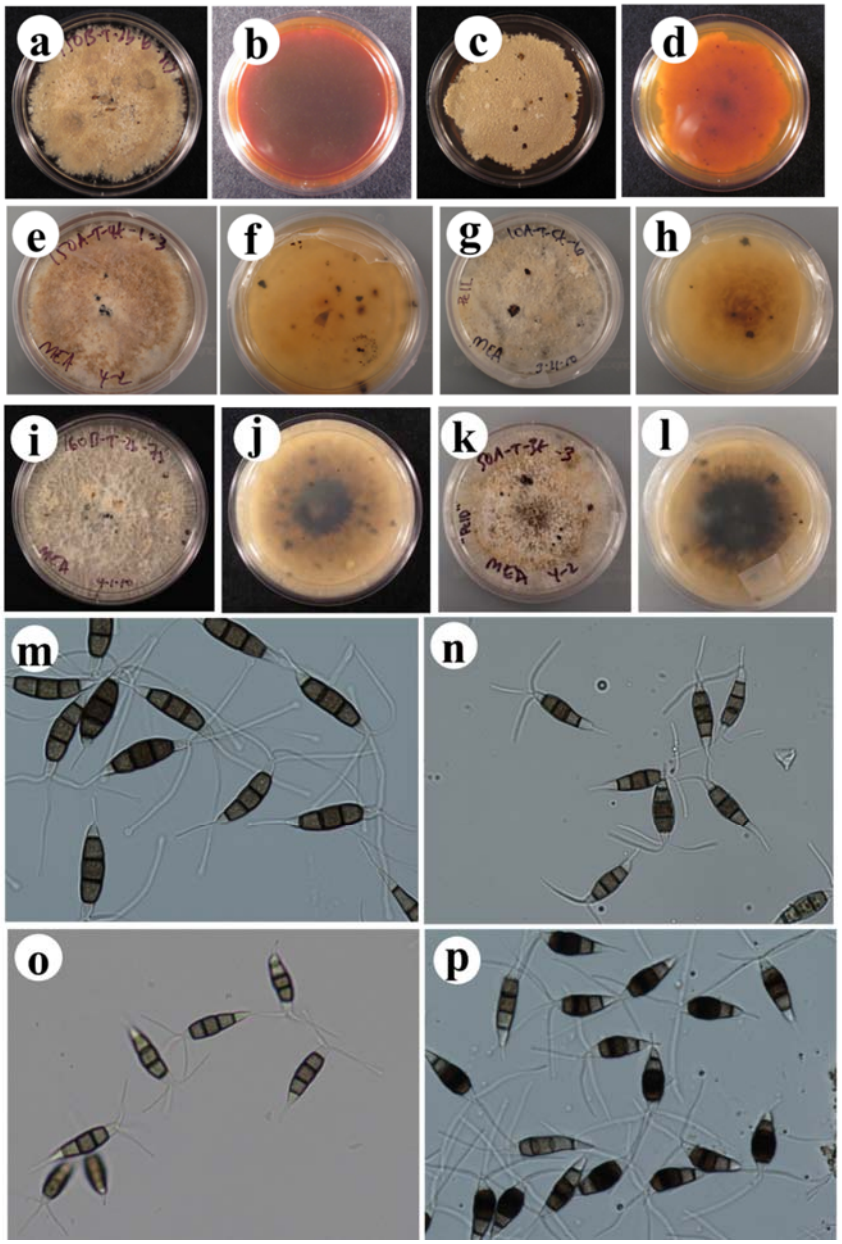

Fig. A-9. *Pestalotiopsis* morphotypes common as greenbrier epiphytes

Colonies on Difco MEA, 100 mm plates; conidia here generated via autoclaved greenbrier stem segments in wet chamber: **(a-d, m)** *Pestalotiopsis* sp. 1 (sp. code: Pe4), 2 isolates, top and bottom, and conidia, **(e-h, n)** *Pestalotiopsis* sp. 2A (sp. code: Pe1L), two isolates, top and bottom, and conidia, **(i-l, o)** *Pestalotiopsis* sp. 2B (sp. code: Pe1D), two isolates, top and bottom, and conidia, **(p)** *Pestalotiopsis* sp. 3 (sp. code: Pe2), colony image unavailable for this morphotype, characterized by clean (no yellow) white hyphae with concentric rings of black oozing conidia;

Notes: Only Difco MEA produced characteristic hues seen in Pe1L vs. Pe1D - another brand of MEA tested did not. The "L" of sp. code Pe1L refers to the bright, luminescent quality of the exudate when the plate is viewed from below compared to the dim, dull, or pale color of Pe1D. The division of Pe1L and Pe1D was well supported by ITS sequences (Ch. IV).