

The Pennsylvania State University

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FUNGAL COMMUNITY ECOLOGY OF LYCORMA DELICATULA IN
PENNSYLVANIA, AND THE SOCIAL-ECOLOGY OF BIOLOGICAL INVASION
RISK

A Dissertation in

Entomology and in International Agriculture and Development

by

Mariam B. Taleb

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The dissertation of Mariam Taleb was reviewed and approved by the following:

Julie M. Urban
Associate Research Professor of Entomology
Dissertation Co-Advisor
Co-Chair of Committee

Ed Rajotte
Professor of Entomology
Dissertation Co-Advisor
Co-Chair of Committee

Tanya Renner
Assistant Professor of Entomology

Leif Jensen
Distinguished Professor of Rural Sociology and Demography

Gary Felton
Professor of Entomology
Head of the Department of Entomology

ABSTRACT

The incidence of biological invasions is and has been rising with no evidence of a coming plateau (Seebens et al., 2017). While the direct impacts of biological invasions are reason enough for concern, the interactions of biological invasions with some of the most critical global ecological and social concerns we face today—anthropogenic climate change, biodiversity loss, and global food security—make the issue all the more urgent. The metrics by which we define and measure the incidence and impacts of invasive organisms are under debate in the literature, but it is widely agreed that once established in a new range, introduced species, through trophic interactions, competition and hybridization are economically and ecologically costly. Mitigating the impacts of the growing incidence of invasive organisms requires development of avenues of inquiry into the determinants of success and modes of impact for any one invasion, and for the global rise in invasions. Moreover, these new modes of inquiry demand interdisciplinarity in theoretical and empiric studies.

Spotted lanternfly or *Lycorma delicatula* (Hemiptera: Auchenorrhyncha) is an invasive pest of over 70 woody-stemmed plant species, especially grapes and timber trees, first detected in southeastern Pennsylvania in 2014 (Barringer et al. 2015). *L. delicatula* is a large-bodied, voracious phloem-feeder and its feeding damage can cause loss of vigor, wilting, and reduced overwintering success. In addition, it deposits sugary waste, or honeydew, on the surfaces of host plants of *L. delicatula* as well as surrounding vegetation, which facilitates the growth of sooty mold. Sooty molds, a polyphyletic group

of fungi, colonize honeydew deposits and form thick black mycelial mats on substrates (Hughes & Seifert 2012). Sooty molds are not direct plant pathogens, but they can stress host plants by reducing their photosynthetic capacity by covering leaves, increasing heat stress, and preventing normal transpiration (Chomnunti et al. 2014). Here, I use emergent methods in mycology to describe the effect of *L. delicatula* on plant surface fungal communities, and to examine the component fungal taxa of sooty molds found in association with *L. delicatula*.

In **Chapter 2**, I used DNA amplicon sequencing to describe the fungal community structure on adaxial leaf surfaces of *Ailanthus altissima* trees (Tree-of-Heaven) exposed to *L. delicatula* honeydew over one season's feeding. I found that fungal communities became richer and more similar to one another as they were exposed to honeydew. While it is expected for any deciduous leaf microbial community to become richer over time, it is uncommon for those communities to become more similar, indicating an effect of the honeydew or *L. delicatula* as opposed to just an effect of time. I also found evidence that a species of *Trichomerium* fungus was associated with sooty mold development on all sampled plants.

In **Chapter 3**, I used methods similar to those in Chapter 2 to examine fungal community structures on leaf surfaces of wild and cultivated grapes, *Vitis riparia* and *Vitis vinifera*. I found that fungal community composition differed between the two *Vitis* species. Diversity slightly decreased with exposure to *L. delicatula* in both *Vitis* species, but I did not find evidence of communities becoming more similar to one another, either within or between plant species. I also found two fungal genera which were associated

with sooty mold, *Trichomerium*, which was significantly more abundant in *V. riparia*, and *Cladosporium*, which was nominally more abundant in *V. vinifera*, though not significantly. *Trichomerium* was far more abundant across the breadth of samples than *Cladosporium*, indicating that while multiple taxa may be found in sooty mold facilitated by *L. delicatula*, *Trichomerium* may have a stronger association with the insect pest.

In **Chapter 4**, I sought to expand on findings from Chapters 2 and 3 by complementing molecular data with morphological data examining the ultrastructure of field-collected sooty mold-infested leaf samples from plants actively fed upon by *L. delicatula*. While sooty mold colonies on collected leaves were morphologically mixed, I found characters from an apparent morpho-species of *Trichomerium* on every collected sample. The *Trichomerium* morphospecies was abundant on most samples, but more dominant within admixtures of sooty mold on samples with less severe sooty mold, and samples from wild-type plant species. Most sooty mold characters other than those of the *Trichomerium* morphospecies resembled those of species in the order Capnodiales but appeared to represent multiple families and genera within it.

These findings validated my hypothesis that from **Chapters 2 and 3** that while sooty mold communities in association with *L. delicatula* may be mixed, there is an apparent *Trichomerium* sp. which, based on the literature, is uncommon in similar studies in this region, yet commonly found when *L. delicatula* is present and feeding. A better understanding of the microbial ecology of *L. delicatula* may inform future efforts to develop monitoring and management tools for *L. delicatula* and its associated sooty mold.

Biological invasion is definitionally a human-mediated phenomenon—it is the role of economic and social behavior of humans instigating biological invasion which sets it apart from other forms of range expansion. However, the process of biological invasion and the factors which govern its success or failure and its ecological and economic impacts have not been addressed in socio-economic terms in the literature. Meeting the challenge of the growing incidence of bioinvasions demands that socio-economic factors in biological invasion be defined and examined.

In **Chapter 5**, I conducted an integrative review of literature regarding ecologically unequal exchange theory, a sociological theory which posits that countries with economic and political power have undue access to natural resources, and literature regarding biological invasion. From the findings of the literature review, I proposed a conceptual model which argues that due to political and economic inequality, at every stage of biological invasion, the risk of incidence and impact unequally falls on the least developed countries, whose capacity to prevent, manage, and mitigate biological invasions is limited by the same political and economic inequality.

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DEDICATION

To Matt, with love

Finally. Now onto the next adventure, together.

EPIGRAPH*Lost*

Stand still. The trees ahead and bushes beside you
Are not lost. Wherever you are is called Here,
And you must treat it as a powerful stranger,
Must ask permission to know it and be known.
The forest breathes. Listen. It answers,
I have made this place around you.
If you leave it, you may come back again, saying Here.
No two trees are the same to Raven.
No two branches are the same to Wren.
If what a tree or a bush does is lost on you,
You are surely lost. Stand still. The forest knows
Where you are. You must let it find you.

--David Wagoner (1999)

Chapter 1

Introduction

The incidence of biological invasions is and has been rising, with no evidence of a coming plateau (Ricciardi 2007; Seebens et al. 2017). The metrics by which we define and measure the incidence and impacts of invasive organisms are under debate in the literature, but it is widely agreed that once established in a new range, introduced species, through trophic interactions, competition and hybridization can cause incredible ecological damage (Ignace et al. 2018; Kenis et al. 2009). Bioinvasions are also economically costly, primarily threatening agricultural production, human disease management, and built infrastructure (Bradshaw et al. 2016). While the direct impacts of biological invasions are reason enough for concern, the interactions of biological invasions with some of the most critical global ecological and social concerns we face today—anthropogenic climate change, biodiversity loss, and global food security—make the issue all the more urgent (Early et al. 2016; Paine et al. 2016).

At its best, invasion ecology seeks to define and describe the factors influencing each stage of biological invasion as well as the impacts and opportunities for intervention at those stages. Unfortunately, bioinvasions are too often described using terms with muddled definitions, imbued with value systems and cultural perspectives. Often these terms are used in way that imply biological invasion is a binary process—either a population is invasive, or it is not (Warren 2007). However, biological invasion is a complex non-linear stepwise process (Blackburn et al. 2017). Scholars often express

concern in the literature that implying distinct binary boundaries between the natural and nonnatural flattens ecological and political-economic textures surrounding biological invasion (Colautti & MacIsaac 2004; Stoett 2010; Tassin & Kull 2015; Warren, 2007).

Good management of biological invasion demands nuanced management of ecological risk and political risks (Larson et al. 2011). Yet unclear terms permit the biological invasion broadly and specific bioinvasions to be framed in ways advantageous to their describers, either intentionally or unintentionally (Stoett 2010). This can serve to exclude stakeholders whose relationships to bioinvasions do not fit within the dominant framing. For example, bioinvasions may cause greater economic damage to a small group of stakeholders relative to their personal incomes, and cause lesser greater economic damage to a much larger group of stakeholders—where should mitigation efforts be focused? (Holmes et al. 2017) These are political questions as much as they are ecological and are deeply influenced by terminology and framing. Framings which create a sense of urgency around specific bioinvasions and the industries they threaten can also serve to depress more wholistic ecological approaches in favor of targeted research (Burgiel 2020; Pearson et al. 2018; Robertson et al., 2020). But excessively targeted foci can fail to capture impacts beyond their scope, and fail to inspire creative monitoring, prevention, and management tactics (Tassin & Kull 2015).

To evade and overcome the ontological problems of invasion ecology, it has been suggested that the field work to interpret individual bioinvasion as ecological phenomena with complex contexts, and work to conduct research and management programs with that in mind. To that end, in this dissertation, the bioinvasion of *Lycorma delicatula* into the eastern United States is investigated as an ecological phenomenon by exploring its

fungal ecology. It has also been suggested that invasion ecology would be more capable of constructing informative models if a greater effort were made to fully incorporate more sociological analyses into models. In this dissertation, the process of biological invasion is then also analyzed in a socio-ecological framework, in order to understand how the human mediation of biological invasion may explain the paradoxes and gaps in invasion ecology theory.

***Lycorma delicatula* (Hemiptera: Fulgoridae)**

Lycorma delicatula (Hemiptera: Fulgoridae), or Spotted lanternfly, is a bioinvasive insect which it native to China, Vietnam, Japan, and India. Prior to its introduction in the United States, *L. delicatula* was reported to have invaded South Korea, where it was first detected in 2004 (Han et al. 2008). In S. Korea, *L. delicatula* was reported to be a pest of fruit crops, especially grapes and tree fruits, and a nuisance pest in built environments (Park et al. 2009; Song 2010). The insect was first detected in the United States in 2014, in southeastern Pennsylvania (Barringer et al. 2015). Because it had previously known to cause economic damage in Korea, there was a quick effort by state and local regulatory agencies to contain and eradicate the population (Urban et al. 2021). However, at the time of detection, entomologists from the Pennsylvania Department of Agriculture were able to collect more than 100 specimens in a single day (Barringer et al. 2015). This may indicate that either the initial propagule population was large, or that *L. delicatula* had been introduced for some time before detection. Either case would help to explain why eradication was not successful. Since 2014, *L. delicatula* has spread and established ten

additional states in the northeast United States, and been detected in several more (Eshenhaur, 2022).

L. delicatula feeds on the phloem of over 70 known plant species, across a breadth of landscapes which make it a threat in nearly every context—forestry, agriculture, ornamental and built environments. *L. delicatula* tend to feed in large numbers on individual plants, causing wilting, branch dieback, and even oozing wounds in trunks of trees (Barringer & Ciafré 2020). In grape vines, which have been particularly affected by *L. delicatula*, phloem-feeding by the insect can reduce overwintering success, vine growth, and crop yield (Song 2010; Urban et al. 2021). In wild plants, the effect of feeding has not been well established in ecological terms but has been projected to have the potential to reach \$236 million in economic losses to the timber industry in Pennsylvania alone (Harper et al. 2019). These impacts account only for direct damage from *L. delicatula*. What has received less attention in the literature is the effect of indirect damage, including the facilitation of sooty mold.

Management efforts in each of these landscapes has been hindered by the diversity of landscape sin which *L. delicatula* must be managed, and their late-season mobility. Beginning in late August and early September, adult *L. delicatula* begin to fly in large numbers to previously uninfested areas (Mason et al. 2020). In vineyards, for example, adjacent forests can harbor far larger populations which can move into grape vines (Leach & Leach 2020; Wolfen et al. 2019). *L. delicatula* therefore demands management plans which take into account the contexts of not only the target landscapes, but all those adjacent to it as well, complicating the effort to develop strong monitoring programs or effective mechanical, cultural, and chemical control plans.

It is estimated that to decrease the population of *L. delicatula*, rather than just slow its growth, 35% of all *L. delicatula* individuals would have to be treated with a combination of the few effective control methods across all relevant growth stages (Strömbom & Pandey 2021). Because of this, and their mobility, while early detection is critical to slowing the spread of any bioinvasive population, which is especially true of *L. delicatula*. However, monitoring has proved difficult, despite efforts to leverage collaborations and innovations to overcome barriers to success. Visual searches for egg masses are limited in newly infested areas where egg masses may still be sparse because of the high labor costs to search any and all hard surfaces for them (Liu et al, 2020; Urban et al. 2021). Alternatives have included behavioral traps for nymphs, but no attractants have been found to increase efficacy in sparsely population regions (Francese et al. 2021; Nixon et al. 2020). More creative ideas have included dogs trained in detecting egg masses (Essler et al. 2021), infrared imaging to seek adults (Liu et al. 2021), and environmental DNA (eDNA) (Allen et al. 2021). eDNA has been promising, in that the probability of detection using eDNA has been much higher than that of visual detection. Given the spatial unpredictability of *L. delicatula* at all life stages, there is reason to continue to seek monitoring tools which do not require the direct detection of the insect.

Sooty mold

Sooty molds are a polyphyletic group of fungi which form superficial mycelial mats on their plant and non-plant substrates by utilizing sugary depositions in the environment,

most often honeydew (Chomnunti et al., 2014; Perez et al., 2009). Amber fossils indicate that sooty molds in Capnodiaceae have a long history as slow-growing epiphytic saprophytes, and extant sooty molds seem to live this way when honeydew is unavailable, though none are known to directly parasitize their substrates (Hughes & Seifert, 2012; Schmidt et al., 2014). The definitive characteristic of sooty molds is the exhibition of the sooty mold lifestyle, and the adaptations required to survive it, including melanin depositions in their cell walls which protect hyphae from UV exposure, and hyphae with mucilaginous outer walls and form entwined mycelial mats in order to absorb and hold onto water in low water activity environments (Chomnunti et al. 2014).

Sooty mold which is facilitated by *L. delicatula* can cause compounding damage to the health of plants already under the stress of herbivory. Sooty molds can prevent photosynthesis, though effects vary in relation to a plant's total photosynthetic capacity, and potential for plasticity (Insausti et al., 2015). Sooty mold can also cause water and heat stress on heavily infested leaves by interfering with stomata while black mycelia increase the heat retention of the phyllosphere (Hughes & Seifert, 2012). The impact of sooty mold on the community in the phyllosphere is not well known, though fungal community diversity effects have been suggested (Menkis et al., 2015), which might imply direct and indirect knock-on effects to plant health. Sooty mold is also of concern for its impacts on economic activities, as it affects the market value of crops, and in the case of SLF, where prevalence on non-plant substrates is high, the value of homes and property (Hughes & Seifert, 2012; Urban, 2019). Further, there is some evidence of potential impacts to human health, especially when sooty mold is present in high concentrations. For example, sooty molds have been shown to accumulate environmental

pollutants on leaf surfaces, and to cause allergic reactions (Guarneri et al., 2008; Jouraeva et al., 2006).

The specificity of causal taxa in sooty molds is not known, and highly debated. Hughes' (1976) observations indicated that sooty mold taxa are mixed on substrates and exhibit no specificity. The unspecific sooty mold hypothesis argues that fungi adhere to the Baas-Becking axiom that “everything is everywhere, but the environment selects,” and that sooty mold dispersal and colonization is an entirely stochastic process. In later studies of pest complexes, specificity has been shown and rejected across various contexts and methods (Insausti et al., 2015; K. W. Kim, 2016). However, most sooty mold complexes are studied in contexts of a singular insect pest and singular plant host, obscuring the role of insect pest species, plant hosts, sooty mold substrates, and/or the nexus of these factors together in creating the selective environmental forces acting on sooty molds.

Given that contemporary interpretations of microbial biogeography indicate gradations of stochastic to deterministic, and contemporaneous to historical factors structuring biogeography of microbes, it is possible that sooty molds do not follow a collective rule of in/specificity (Martiny et al., 2006). Rather, it is likely that the whole community and environment, including facilitating insect pests and the host plants they feed upon, all influence the composition of sooty mold. The composition of honeydew is supportive of this position, in that the composition of honeydew is controlled by many factors, all of which interact with one another. Honeydew is composed of primarily of sugars, but also includes amino acids, minerals, and secondary metabolites derived from plant hosts (Hendrix, Wei, & Lec, 1992; Leroy, Wathelet, et al., 2011; Ramos & Kato,

2012; Wilkinson et al., 1997). The sugars, both their concentrations and forms, have been shown to be determined by insect species, plant host species, diurnal patterns in phloem within plants, and even commensalism with ants (Hendrix, Wei, & Lec, 1992; Hendrix, Wei, & Leggett, 1992; S. H. Taylor et al., 2012; Wilkinson et al., 1997; Zhou et al., 2015). Amino acid and protein content can be influenced by the plant species and phenology, insect species, and interactions with microorganisms (Davidson et al., 1994; Leroy, Wathelet, et al., 2011; Sabri et al., 2013). Secondary metabolites are determined by the immediate concentrations in plant hosts, and the life history of insect pests and their interactions with parasitoids and microorganisms (Buitenhuis et al., 2004; Dhimi et al., 2011; Leroy, Sabri, et al., 2011; Ramos & Kato, 2012; Schwartzberg & Tumlinson, 2014b; Vandoorn et al., 2015). Considering the diversity of honeydew composition, and the complexity of factors structuring it, it is reasonable to expect complex ecological factors structuring the causal taxa in sooty mold complexes.

L. delicatula poses an opportunity to practice more wholistic approaches to bioinvasion management. Sooty molds, though deemed less critical in vineyards in the United States than initially feared, should be investigated from a mycological perspective because the full ecological impacts of *L. delicatula* are still not known, and there is potential for sooty mold to cause unexpected damage in landscapes which have received less research attention to date, natural forests included. Moreover, should sooty mold, as it relates to *L. delicatula*, be identified, it may pose an opportunity to develop additional monitoring tools which do not require detecting the insect itself, but rather an environmental signature of its recent presence.

Ecologically Unequal exchange

Ecologically Unequal Exchange (EUE) is a political-ecological examination of the power imbalances between nations which shape their ecological relationships, especially regarding the capacity of nations to extract natural resources and externalize environmentally detrimental waste (Jorgenson, 2016). Further, EUE describes responsibility for ecological damage not in terms of production but in terms of consumption, arguing that nations with high wealth accumulation disproportionately control demand and pernicious production (Muradian et al. 2002).

EUE's origins can be found in a series of novel approaches to the nature/society divide which emerged in the 1970s-1980s from political-economic critiques of development theory, namely world systems theory (Bassett & Peimer, 2015). Immanuel Wallerstein proposed world systems theory (WST) (1974) as a framework to examine the world-economy as a global division of labor among nations, forming three distinct blocks: the core, the semi-periphery, and the periphery. The core produces high value, low risk products, while the periphery is exploited for natural resources and other high-risk low value products (Chase-Dunn & Grimes, 1995; Wallerstein, 1974, 1976). WST is often applied to EUE to frame the forces that maintain the relational stances of nations in the context of environmental harm, given increasing gestures of intent to protect global ecologies (Bassett & Peimer, 2015). In other words, ecological relationships among the

periphery, semi-periphery and, and core are created and reinforced by the same structural dynamics that form the world-economic system.

EUE literature has largely relied on quantitative methodologies to show inequalities, but this has limited its applications (Stone-Jovicich 2015). EUE scholars have noted that the theory would be strengthened by methodologies which would make it more accessible and applicable in ecology and environmental sociology alike (Jorgenson, 2016; Stone-Jovicich, 2015). In the case of biological invasion, an issue for which there is insufficient global data (Pyšek et al. 2008), a treatment of EUE as a part of WST, and applying both in tandem would offer the methodological strengths of WST to the specific focus of EUE.

EUE is an apt framework to apply to biological invasion in order to better understand at the global scale what socio-economic factors may interact with the process of biological invasion, and how inequality may influence those interactions, or our perceptions of them (Givens et al. 2019, Jorgenson & Clark 2009). This is particularly true given that EUE has so often been used to frame the ecological phenomena which are known to interact with biological invasion, including biodiversity loss and habitat destruction (Jorgenson 2010; McKinney et al. 2010; Moran et al. 2013; Rice 2007; Shandra et al. 2010). It is possible that the same forces affecting these other forms of ecological damage would affect biological invasion, and that the unequal distribution of these other ecological harms would also amplify inequality in biological invasion by nature of their interrelations.

Biological invasion is a growing crisis, and our current models of responding to it are not working—we must be self-critical and creative in this time. Instead of simplistic definitions of bioinvasions, efforts should be made to fully use wholistic models of biological invasion to describe the state of each bioinvasion in its entirety, including known and hypothesized ecological and political-economic contexts (Vaz et al. 2017). In this dissertation, I model new ways to approach an individual bioinvasion, and invasion ecology at large.

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Chapter 2

Fungal community succession in the phyllosphere of *Ailanthus altissima* colonized by spotted lanternfly (*Lycorma delicatula*, Hemiptera: Auchenorrhyncha) in southeastern Pennsylvania.

*An amended version of this work is under review: Taleb, Mariam; Henderson, Sarah; Urban, Julie. Fungal community assembly and succession in the phyllosphere of *Ailanthus altissima* colonized by Spotted lanternfly, *Lycorma delicatula*, in southeastern Pennsylvania. *Agric. For. Entomol.*

Spotted lanternfly, *Lycorma delicatula*, is an invasive insect pest of fruit crops and forestry in the mid-Atlantic United States. *L. delicatula* directly harms its more than 70 host plants by feeding on their phloem, and indirectly harms its host plants through the deposition of honeydew in the phyllosphere, which facilitates sooty mold growth. The effect of indirect damage by *L. delicatula* on the diversity and structure of phyllospheric fungal communities has not been explored. We sampled the adaxial leaf surfaces of *Ailanthus altissima* exposed to *L. delicatula* honeydew accumulation on a highly infested residential property. We then used amplicon sequencing of the ITS1f-ITS2 gene region to assess fungal community structure, and to seek successional patterns. Variance of fungal community assemblages significantly decreased over time, and a statistically insignificant increase in richness was observed. Results indicated no effect of sooty mold on fungal community, but a small, yet significant effect of time. Evidence of facultative

successional patterns was shown. A potential causal taxon for sooty mold, *Trichomerium*, was also identified.

Introduction

Invasive organisms pose high, and growing, economic and ecological risk to forestry and agriculture (Seebens et al. 2017). Economic costs are driven by the loss of salable material by death or unacceptable damage to crops and timber and by the cost of preventative, protective, and mitigative interventions (Paini et al. 2016; Stenlid et al. 2011). In 2000 it was estimated that in the United States, 25% of agricultural productivity was lost to invasive organisms valued at \$58B, and that direct costs of interventions (a potential underestimate) cost an additional \$4B (Ziska et al. 2011). Death and damage of crops and timber trees can cause devastating ecological damage as well. Loss of trees can have cascading effects on the local ecosystem, altering trophic relations and nutrient cycles and reducing resilience to weather events and other disturbances (Stenlid et al. 2011). Similar to examples of economic damage, management tactics can create as much ecological damage as the organisms themselves (Simberloff 2014). More precise and efficient monitoring and intervention tactics, therefore, are and will be critical to combatting invasive organisms and their impacts.

Little is understood about what drives vulnerability or resistance to invasive organisms, though a better understanding of these dynamics is critical to the development of effective and precise management tools. Critiques of invasion ecology suggest that this gap is the result of misplaced attention to the characteristics of successful invaders, rather

than the characteristics of successful invasion processes (Keller et al. 2011). In recent years, research in invasion ecology has sought to rectify this by introducing models of invasion dynamics which integrate whole-ecology perspectives on the incidence and impact of novel organisms (Blackburn et al. 2011; Colautti & MacIsaac 2004; Jeschke et al. 2013). One way these frameworks could be applied is to inform research about patterns of ecological communities exposed to invasive organisms.

Though the microbial world is often rendered invisible, interactions with it can have enormous ecological effects. Phyllospheric communities, for example, mediate and are mediated by the pests and pathogens most threatening to agricultural and forest systems (Stone et al. 2018; Stone & Jackson 2020). Microbes can alter the attractiveness or palatability of their tissues to herbivores (Taylor et al., 2015), or serve as attractants to natural enemies of insect pests (Bennett et al. 2016; Perez et al. 2009). They can prime plants to defend against insect herbivores or plant pathogens, and, through cross-talk, suppress these or other defenses (Humphrey et al. 2014). Microbial communities can also compete against plant pathogens, serving as an indirect form of resilience (Taylor et al. 2015). Conversely, plant pathogens can alter the structure of phyllospheric microbial communities, and invasive insects can shape the communities in the phyllosphere by altering the environment or transporting new microbes to the environment (Dhami et al. 2013).

Phloem-feeding insects, such as spotted lanternfly (*Lycorma delicatula*, Hemiptera: Fulgoridae), alter phyllospheric and broader ecology through the deposition of honeydew. The release of sugars via honeydew is critical to the maintenance of osmotic pressure within phloem feeders, making honeydew a harsh environment for

water-limited microbes (Douglas 2006; Fisher et al. 1984). Honeydew depositions have been shown to alter carbon cycles (Santos et al. 2013), manipulate plant defense (Schwartzberg & Tumlinson 2014), and alter fungal communities in the phyllosphere (Menkis et al. 2015). In many instances, honeydew facilitates the growth of sooty mold, a polyphyletic group of fungi adapted to low water activity, and high UV exposure which form thick mycelial mats on honeydew-coated surfaces, including leaves.

Lycorma delicatula is an invasive insect native to China and Taiwan, first detected in southeastern Pennsylvania in 2014, and since that time, has spread to establish known populations in 11 states in the eastern US (Urban et al. 2021). As a voracious generalist, with more than 70 known woody-stemmed plant hosts in the region (Barringer & Ciafré 2020), *L. delicatula* is a threat to fruit crops such as grape vines and tree fruits, ornamental trees such as black walnut and maples, as well as common successional trees (Urban 2019). Its primary host, *Ailanthus altissima* is a naturalized invasive tree species first introduced to the eastern United States in 1748 via Europe, though it is in China, and is shared with *L. delicatula*'s native range (Ding et al. 2006).

Insufficient attention has been paid to the potential indirect impacts of *L. delicatula* honeydew. It is unknown how honeydew affects the local and microbial ecology, other than that it appears to facilitate sooty mold. Even so, it is not known what factors in the honeydew or local ecology determine the symptomology, distribution or severity of sooty mold outbreaks (Song 2010; Urban 2019).

In this study, we used high throughput amplicon sequencing (HTS) of the internal transcribed spacer region (ITS), specifically the ITS1f-ITS2 gene region ('EMP.kabir'

primers (Thompson et al. 2017)) to explore the community structure of fungal epiphytes on *A. altissima* exposed to *L. delicatula* over the course of the 2017 season in southeastern PA. We hypothesized that HTS could be used to assess the effect of *L. delicatula* exposure on the fungal community in the phyllosphere of *L. delicatula* hosts, trends in changes to the fungal community structure over time, and what those trends indicate about patterns of succession in this system. We expected that the individual tree sampled would be the strongest indicator of community structure, but that communities would become less dissimilar over time, as they are exposed to similar disturbances. We also expected that species richness would positively correlate with time as microbial colonizers adapted to honeydew, specifically sooty mold fungi, digest the honeydew and in doing so, develop new, less harsh niches, and that total fungal diversity would negatively correlate with time, as honeydew continued to disturb the habitat, allowing best-adapted taxa to thrive. Additionally, we expected that communities with visibly detectable sooty mold would be more similar, as honeydew accumulated and selected for fungi capable of thriving in it, and that diversity and richness will negatively correlate with detections of visible sooty mold, as sooty mold dominates the community. The characterization of fungal community assembly under the pressure of *L. delicatula* will explore the fungi causing sooty mold, and ultimately inform integrated pest management of both *L. delicatula* and the sooty mold it facilitates, potentially by suggesting a fungal signature that could be sought to monitor for *L. delicatula*.

Methods

Sampling

Samples were collected at a residential property with no known history of pesticide use in Berks Co., PA (40.40°N, 75.71°W) over approximately nine weeks from August 3 - October 4th, 2017. During the sampling period, nine sampling events occurred, typically every six to nine days (Table 2-1). The sampled property is located approximately four miles from the site at which *L. delicatula* were first detected in Pennsylvania. Five individual *A. altissima* trees were selected from along a boundary edge between managed lawn and forested land, where this species is most typically found. Branches at approximately 1-2m height were selected and marked with hanging tags attached with fishing wire to identify their location over the course of sampling. The method was selected identify sample tree location to minimize interference with *L. delicatula* by visual or chemical stimuli. At the time of each sampling event, the continued presence of *L. delicatula* was confirmed for each tree, and observations of visible sooty mold development were recorded for each branch. We had developed a five-point scale for the rating of sooty mold severity, but in all selected trees, the severity was never greater than a "1". For each tree, fungal communities on the adaxial surface of plant leaves were sampled with sterile cotton swabs, and the surface of the same branch was sampled throughout all sampling events for this study. Care was taken to swab across the entire surface of each leaf, and to use enough pressure to dislodge epiphytic cells without visibly damaging leaf surfaces. Swabs were then transported to the laboratory and stored at -20°C. In some instances, trees or selected branches wilted or died from feeding

damage by *L. delicatula*. In these instances, no samples were taken from that individual branch for any weeks following death.

Table 2-1: Sample collection observations. For each sample collection date, the number of plants sampled with observed *L. delicatula* and observed sooty mold is noted.

	3-Aug	9-Aug	18-Aug	24-Aug	31-Aug	8-Sep	14-Sep	25-Sep	4-Oct
<i>L. delicatula</i> infestations ¹	5	5	5	5	5	5	5	5	5
Sooty Mold Observations ²	0	0	2	3	3	5	3	4	4

¹Even in samples where selected branches had died, because feeding observations related to whole plants, n=5 in all collection dates. ²Due to the death of selected branches from feeding damage, for collection dates August 3rd- September 8th, n=5, but for collection dates September 14 and September 25, n=4, and on October 4, n=3.

DNA Extraction, amplification, and sequencing.

DNA extraction was performed using the DNeasy PowerSoil Kit (QIAGEN USA, Germantown, MD), according to the manufacturer's protocols for swabs with polymerase chain reaction (PCR) inhibitors. For each sample and for three water controls, according to the Earth Microbiome Project ITS protocols, the ITS1 gene region was amplified by PCR with primers ITS1f-ITS2 (EMP.ITSkabir) using the Platinum Hot Start 2X Master Mix kit (ThermoFischer Scientific, Santa Clara, CA) (Thompson et al. 2017). Sample-specific barcodes were then attached to PCR products, and barcoded triplicate products were then pooled by sample. Amplicons were purified using ExoSAP-IT™ (ThermoFischer Scientific, Santa Clara, CA). Barcoded and pooled samples and amplified water controls were sent to the Genome Sciences Facility at the Penn State College of Medicine (Hershey, PA) for sequencing on the Illumina MiSeq platform.

Bioinformatics

Initial quality filtering and processing of raw sequences was done using the QIIME pipeline (Caporaso et al. 2010). De novo operational taxonomic units (OTUs) were picked at a default clustering threshold of 97%, and reference sequences were randomly selected from clustered OTUs. OTUs were then rarified to 31,387 sequences per sample to remove heterogeneity of sequencing depth. OTU tables were then exported, and singletons and contaminants (OTUs for which in all samples, the number of sequence reads <2x the sequence reads in the water controls) were removed (Perazzolli et al. 2014).

To assign taxonomy, the most abundant OTUs, covering 90.00% of non-contaminant, non-singleton sequences, were searched using NCBI Nucleotide BLAST function (Altschul et al. 1990), and taxonomy assignments were hand-curated and annotated. Accessions were limited to culture-based identities, with preference given to accessions from type-material, or to the accession with the highest maximum score, highest query cover, or highest percent identity. Taxonomic identities were assigned at the species level if returned accessions had at least a 97% sequence identity score, at the genus level when accessions had at least a 93% identity score, and at the family or order level given accessions with 90% sequence identity (Menkis et al. 2015). In keeping with the logical stances of the UNITE database, and the methods for including novel literature from the FUNGuild database (Nguyen et al. 2016; Nilsson et al. 2018), taxonomic ambivalence and opacity among fungi were considered and clade-specific protocols were developed based on the most recent literature available. For all analyses, taxonomic

diversity was assessed either by OTU or at the genus level. All other taxonomic levels were only used to inform functional guild assignments.

Lifestyles (defined by FUNGuild as “a group of species, whether related or unrelated, that exploit the same class of environmental resources in a similar way”) were determined for taxa assigned to at least the order level using FUNGuild’s online tool (Nyugen et al. 2016) prioritizing results for the most specific known taxonomic level. If lifestyle data was not available in FUNGuild, it was determined with literature when possible. Similarly, for pertinent lifestyles not noted in FUNGuild (e.g. sooty mold), genera displaying that lifestyle were determined using available literature. In the analysis, only “probable” and “highly probable” results were retained, and each functional group was defined by all of its known lifestyles, i.e. “endophyte-undefined saprobe-plant pathogen” was analyzed as a group separate from “endophyte-plant pathogen” (Nguyen et al. 2016).

Statistics and Visualization

Data analysis was conducted in R (R Core Team 2017) and visualized with ggplot2 package (Wickham, 2016). Bar graphs of mean proportions of sequence counts were constructed to visualize relative abundance by genera and by lifestyles at each collection date. Kendall-Tau correlations were calculated for between all genera and collection date, and between lifestyles and collection date. Chao richness was calculated using the hillR package (Li 2018), and Shannon’s entropy and Simpson index was calculated using the vegan package (Oksanen et al. 2020). Generalized linear models were then fit using the

lme4 package (Bates et al. 2020) to assess diversity over time with “plant” as a random effect (Barge et al. 2019).

A Bray-Curtis dissimilarity matrix was calculated using the R *vegan* package (Oksanen et al. 2020) to estimate differences between fungal assemblages. Non-metric multidimensional scaling (NMDS) was used to visualize pairwise dissimilarities using the *metaMDS* function (Cordier et al. 2012, Humphrey et al. 2014). Non-statistical ellipses were plotted to show the total variance of samples at each collection date. Dispersals (variance) around the medians of samples by collection date were calculated with the *betadisper* function. The *permutest* test function was used to confirm that the variance of dispersals met the assumptions of the *adonis* function. The *adonis* function was then used to perform a PerMANOVA to test the significance of time, and the interaction effect of sooty mold observations. To test for a statistical trend in dispersals over time, a linear mixed model was fit to dispersals (Humphrey et al. 2014).

Adobe Illustrator CC was used to edit design of generated plots (Adobe Inc., 2020).

Results

Taxonomic and Functional Group Identification

Samples were rarified to 31,387 sequences per sample, or a total of 878,836 sequences, represented by 256,698 OTUs. Due to an error in PCR protocols, we struggled to successfully amplify the gene region from samples collected in the first three weeks of

the study. As a result, fewer than three replicates were sequenced for each of the first three weeks, which were therefore excluded from analysis after rarefaction (due to the low number of sequences obtained for these samples).

Table 2-2: Sequencing depths per sample. For each sample, the total sequences count after rarefaction and removal of singletons and contaminants.

	3-Aug	9-Aug	18-Aug	24-Aug	31-Aug	8-Sep	14-Sep	25-Sep	4-Oct
AIL5			26866		26443	27592	27981	26303	26156
AIL6		29682	30778	30193	29357	28068	27630	25721	25044
AIL9	24878			28265		28431	27583		24464
AIL10				24779	22632	24758	25842	25339	¹
AIL11	30358			21522	26436	26404	¹	¹	¹

¹Due to the death of selected branches from feeding damage, no swab was collected. For all other greyed samples, amplification in triplicate was unsuccessful, and the sample was not sequenced.

After removal of contaminants and singletons a total of 6,868 OTUs representing 749,505 sequences were retained (Table 2-2). Following this, taxonomy was assigned for the most abundant OTUs by hand-curation of GenBank BLAST results until a total of 90% of non-contaminant, non-singleton sequences had been searched, represented by 593 OTUs. Of 593 OTUs for which we attempted to assign taxonomy, we successfully determined the phyla of 490 OTU, (89.66% of searched sequences). Interestingly, only 10.34% of BLAST-searched sequences were unable to be identified at all, whereas when using the `assign.taxonomy.py` function of the QIIME pipeline, 34.57% of sequences belonged to OTUs that were unable to be identified. Further, we were able to identify to genus 350 OTUs, or 70.58% of searched sequences using BLAST searches, whereas only 8.25% of sequences were identified to at least genus through the QIIME pipeline. 488 OTUs were identified to at least order and included in the search functional groups conducted using FUNGuild and a literature search. Of these, we identified a functional

group for 477 OTU. Only the 279 OTU whose functional group assignments were considered “Probable” or “Highly Probable” were used in data analyses.

Community Structure: Assembly

All OTU that were assigned belonged to superphylum Dikarya, the majority belonging to Ascomycota (412 OTU, or 81.26% of sequences), and the minority to Basidiomycota (78 OTUs, or 8.40% of sequences). Of Ascomycota, nearly all the sequence abundance was comprised of OTUs from sister clades Dothideomycetes (representing orders Botryosphaeriales, Pleosporales, Capnodiales, Cladosporiales, Dothideales, Mycospherellales, and Venturiales) and Eurotiomycetes (representing the order Chaetothyriales) (Fig. 2-1). Across all high-quality sequences, 81 genera were identified. Kendall-Tau correlations revealed no strong relationships ($> |±0.5|$) between any genus and collection date. The most abundant genera across all samples (by sequence count) were *Aureobasidium*, *Cryptococcus*, *Filobasidium*, *Genolevuria*, *Leptosphaerulina*, *Neosetophoma*, *Phoma*, which are considered cosmopolitan endophytes/epiphytes; *Dothiorella*, *Phyllactinia*, which are known to be miscellaneous plant pathogens; and *Trichomerium*, which is typically found as a sooty mold (Cordier et al. 2012; Quan et al. 2020). Maximum-likelihood models were fit for each of the ten most abundant genera over time, but none were statistically significant.

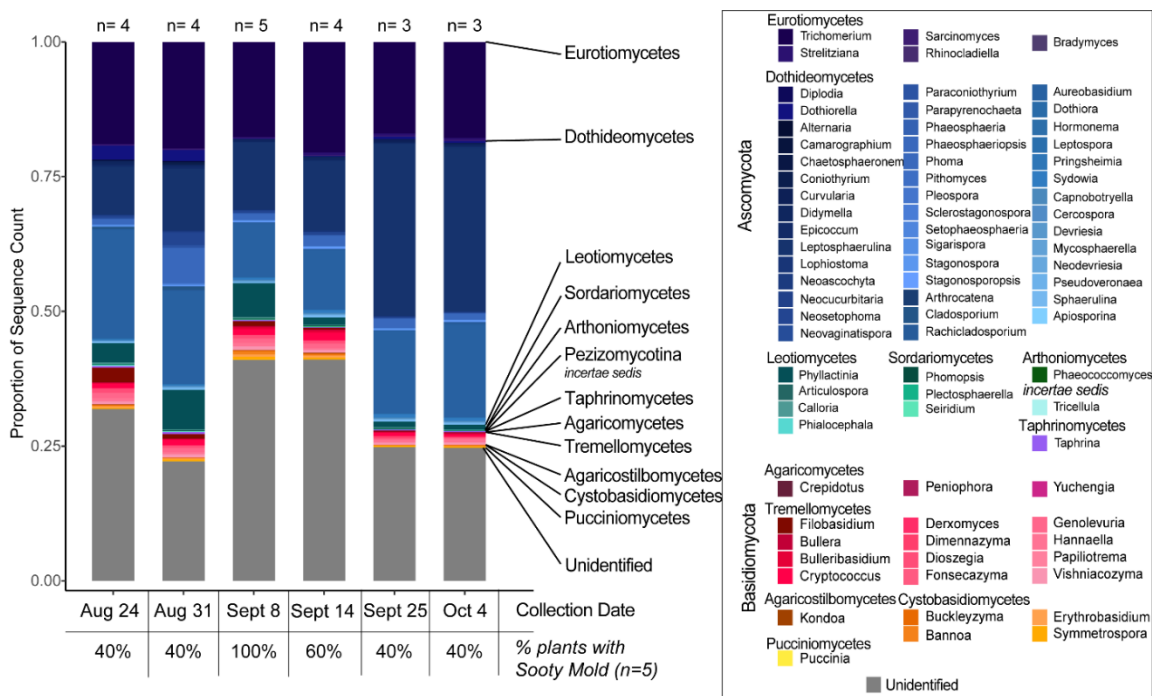


Figure 2-1: Relative abundance of genera by collection date. Comparison of relative taxonomic abundance by mean sequence counts of genera from samples taken at each week. Kendall-Tau correlations between sequence count and week were calculated for each genus, but no correlations were strong ($> | +/- 0.5 |$).

The relative abundances of lifestyles were also visualized by stacked bar graph (Fig. 2-2). The relationship between lifestyles and collection date was assessed with Kendall-Tau correlations, and no groups strongly correlated with time ($> | +/- 0.5 |$). The saprotrophic guild was abundant in all samples. Similarly, the endophyte-sooty mold lifestyle was abundant across all samples, and collection dates.

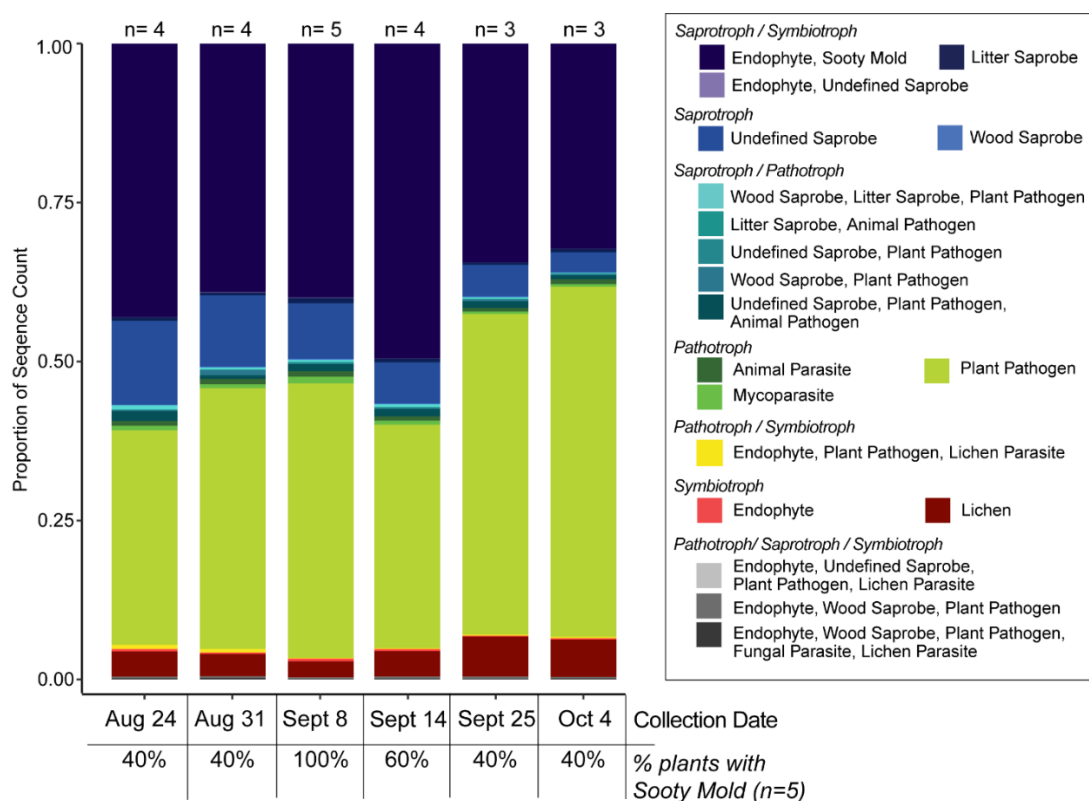


Figure 2-2: Relative abundance of lifestyles by collection date. Comparison of relative functional group abundance by mean sequence counts of functional groups from samples taken at each week. Kendall-Tau correlations between sequence count and week were calculated for each group, but no correlations were strong ($> |+-0.5|$).

Community Structure: Alpha Diversity

Chao richness, Shannon diversity, and Simpson diversity were calculated to reflect the alpha diversity of each sample. Maximum-likelihood mixed linear models were fit to each of the three indices. Though none were significant, Chao richness and Simpson diversity (which prioritizes richness) trended up over time, while the slope of Shannon diversity, which prioritizes evenness, did not (Fig. 2-3).

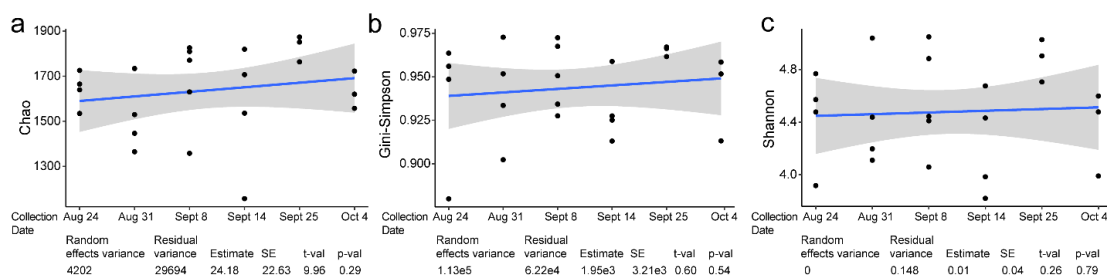


Figure 2-3: Fungal community diversity over time. Points represent individual sample diversity estimates. Line was calculated by method LM. CI=95%. a) Estimated species richness (Chao) b) Change over time in Shannon diversity estimate. c) Community evenness (Gini-Simpson) diversity indices. Maximum likelihood linear regressions were modelled for each diversity estimate, with “plant” included as a random variable to account for repeated measures. No models are significant

Community Structure: Beta Diversity

The dissimilarity between sample fungal assemblages was estimated with a Bray-Curtis dissimilarity matrix. A multivariate analogue of Levene's test for homogeneity of variances showed that the homogeneity of variance between samples from the same week. A PerMANOVA was used to compare fungal community assemblages over weeks. In our model, OTU matrix ~ plant * collection date* sooty mold observations no factors nor interactions significantly affected beta diversity (Table 2-3).

Table 2-3: PerMANOVA of Bray-Curtis dissimilarity indices for fungal assemblages (n=23). 999 permutations were completed, and permutations across “plant” were not permitted in order to account for repeated measures. No models are significant.

	<i>Df</i>	Sum Sqs	F	R ²	<i>p-val</i>
Week* sooty mold (yes/no)	1	0.12	0.78	0.03	0.59
Week	1	0.20	1.29	0.05	0.25
Sooty mold (yes/no)	1	0.19	1.25	0.05	0.25
Residuals	15	2.32		0.68	

¹Due to the death of selected branches from feeding damage, no swab was collected. For all other greyed samples, amplification in triplicate was unsuccessful, and the sample was not sequenced.

An NMDS plot was then constructed to visualize dissimilarities between fungal assemblages of samples (Fig. 2-4). Samples did not appear to cluster strongly by week, but later weeks in the study were more tightly clustered within and between weeks than were samples from early weeks. A maximum-likelihood linear mixed model of variance over time, with plant as a random effect, showed a statistically significant decrease in dispersals around the median over time in the study (Fig. 2-5).

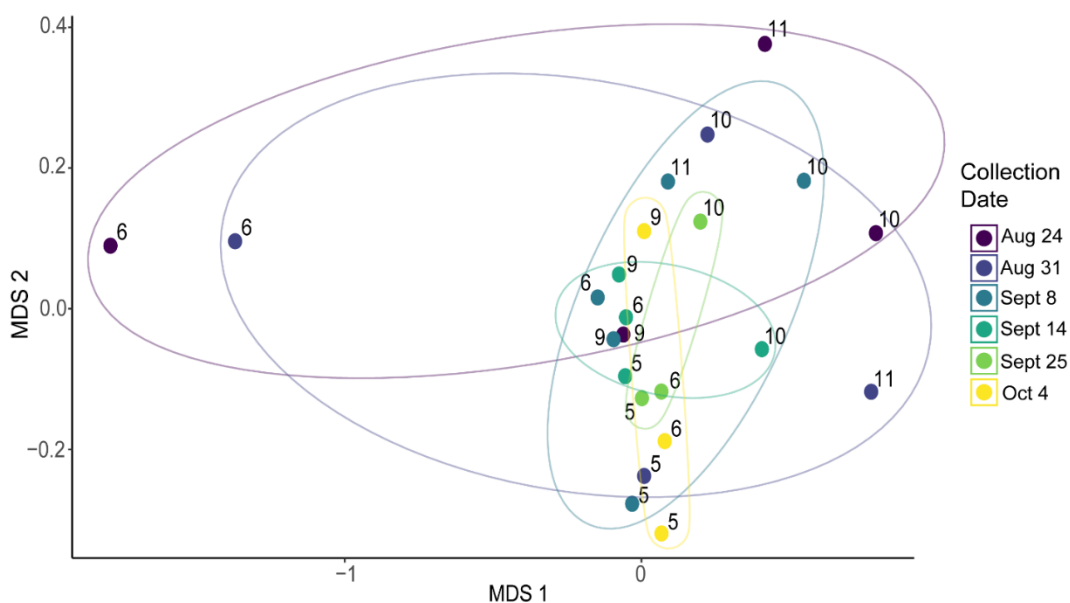


Figure 2-4: Non-Metric Multidimensional Scaling (NMDS) ordinations of Bray-Curtis abundance-based dissimilarities. Each sample is plotted individually and labelled with the individual plant number from which the sample was taken. Points are colored by collection date. Ellipses are non-statistical and represent the spread of all samples at each collection date. Stress =0.06

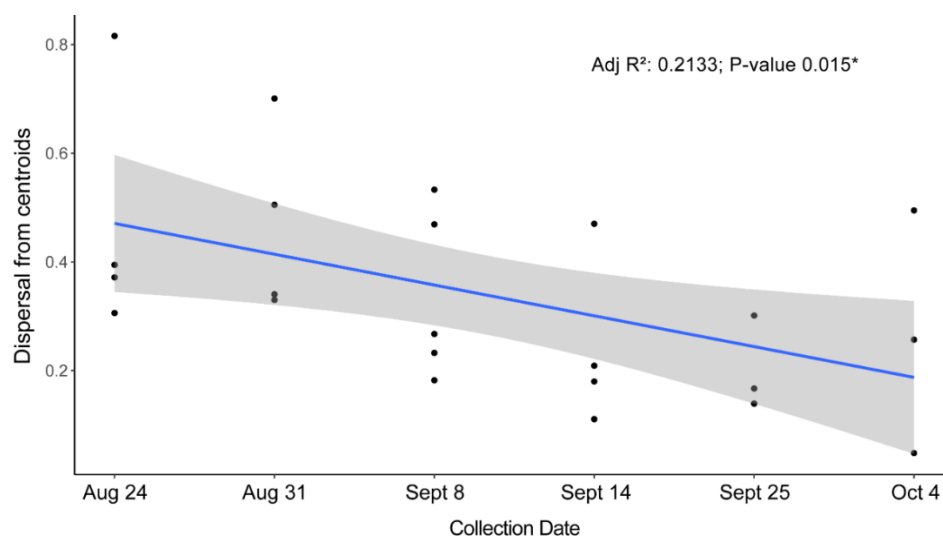


Figure 2-5: Dispersals from centroids over time. Points represent individual sample dispersals from centroids at each collection date, calculated from Bray-Curtis abundance-based dissimilarities. Line was calculated by method “linear model” in ggplot2 (Oksanen et al. 2020). CI=95%. Slope= -0.05; SE= 0.01; df = 20.20; t= -2.53; p=0.01.

Discussion

The goal of this study was to characterize the change in fungal community assembly on leaf surfaces of *A.* over several weeks of exposure to *L. delicatula*. Our results suggested that as honeydew depositions disturbed the phyllospheric community, facultative succession occurred. We found that fungal communities became significantly more similar to one another over time and showed non-significant increase in richness. Our results also indicated that the sooty mold observed in this study may have been caused by *Trichomerium*. Of all genera identified, only two were known to have sooty mold lifestyles, and of these, only *Trichomerium* reached a relative abundance greater than 1% of sequences.

Interpretation of HTS from fungal communities is limited by the body of culture-dependent literature and nomenclatural and phylogenetic resolutions across Fungi. We sought to develop a more thorough method of assigning taxonomy that used the logic of the UNITE and FUNGuild databases but referred to databases with stronger representation of the ITS1f-ITS2 gene region than UNITE currently boasts (Müller & Ruppel 2014; Nguyen et al. 2016; Nilsson et al. 2009). Our taxonomy assignment methods were more successful than expected. We identified 59.02% of all OTUs to at least the genus level, which accounted for 11.8% of all sequences after quality control filtering and rarefaction. There is much more work to be done to align reference datasets with the most contemporary phylogenies, but we found that in the meantime manual curation and annotation of taxonomic assignments can especially strengthen interpretability of fungal amplicon sequencing data.

Though few results were significant, there is some evidence that deterministic processes were captured in this study. Diversity over all (richness and evenness) tends to increase in the phyllosphere over the course of seasons (Laforest-Lapointe & Whitaker 2019). Though neither diversity measure was statistically significant in our study, we found positive correlations for Chao richness and Shannon's Entropy (which prioritizes richness) with time, but a near-neutral slope for Simpson's diversity, which prioritizes evenness. Additionally, we found a statistically significant decrease in variance over time of samples, indicating that fungal communities were becoming more similar over time. Taken in concert these trends indicate the possibility of facultative microbial interactions, in which diverse fungi that are adapted to the consumption of honeydew may be removing a barrier to establishment for other taxa (Chomnunti et al. 2014; Dhama et al.

2013; Koskella 2020; Menkis et al. 2015). This finding is interesting because in the current literature, sooty mold and other consumers of honeydew have been treated largely as competitors in microbial communities (Bruns, 2019).

We found no interaction effect of observations of sooty mold with time on fungal assemblies. However, some results were indicative of a potential identity for the causal taxa of sooty mold in this study. Of all sequences identified to genus, there were only two genera with known sooty mold lifestyles, *Trichomerium* and *Strelitziana*. *Trichomerium* represented 15.21% of all non-contaminant, non-singleton sequences across all samples, and was one of only three genera which represented more than 10% of total sequences. Comparatively, *Strelitziana* represented only 0.16% of all sequences across samples. However, because *Strelitziana* belongs to a recently described family it is unclear whether the ITS2 gene region is sufficient to divide these two genera (Müller & Ruppel, 2014; Nguyen et al. 2016; Nilsson et al. 2009). What is particularly interesting about this finding is that as far as the authors are aware, *Trichomerium* sooty molds have only been isolated from China and Thailand, within the shared native range of *L. delicatula* and *A. altissima* (Chomnunti et al., 2012).

There is not sufficient evidence from this study to identify the causal taxa of sooty mold in this context, but if *Trichomerium* is a causal agent of sooty mold in this system, it could reflect historical biogeography of *Trichomerium*, and a potential recent range expansion, either in being vectored by *L. delicatula*, or by *Ailanthus*, and facilitated by the recent emergence of *L. delicatula* in the US. Future research should seek further evidence of a causal agent in *L. delicatula*-associated sooty mold. These results can be

used in future studies to strengthen culturing methods by providing some *a priori* hypotheses to shape media development.

We often failed to reject the null hypotheses in this study, which may indicate that the pressure of honeydew on the phyllosphere has less of an effect on community assembly than hypothesized and may be a result of the study design. For one, the spatial-temporal scale was intentionally small to restrict our study to biotic rather than abiotic variables. However, it is likely, based on literature that has emerged since the time of sampling that our spatial scale was too restricted (Laforest-Lapointe & Whitaker, 2019). Additionally, we had significant difficulties with amplification in the early weeks of the study due to an error a laboratory procedure, which was corrected in the later weeks of the study.

Ultimately, we chose to include only weeks 4-9 of our sampling in our analysis, because there were fewer than three successful replicates (individual plants) in each of our first three weeks. However, the weeks that were not included in the study represented those that were collected early enough in the season to have very little or no honeydew accumulation. Successional and biogeographical patterns should be examined earlier in the season, and under controlled exposure to *L. delicatula*.

This study was also limited to observations of Spotted lanternfly's preferred host, *A. altissima*, which prevented us from identifying whether these trends correlate with the presence of Spotted lanternfly specifically. *A. altissima* is also not the plant host of greatest economic importance in Pennsylvania, and in fact, it is also an introduced species, which shares its native range with that of *L. delicatula* (Ding et al. 2006). Shared life histories of *Ailanthus altissima* and Spotted lanternfly may have confounded

interpretation of the effects of Spotted lanternfly as an invasive insect. Future studies should compare the effects of Spotted lanternfly on phyllospheric fungal assemblies between plant host species.

Despite high infestations of Spotted lanternfly during the study, and concomitant high depositions of honeydew, we ultimately observed very little sooty mold. Our original field protocols included a five-point scale for sooty mold observations, but we never observed accumulations beyond a “1” on our scale, leading us to record sooty mold instead as visually present or absent. Reports from the *L. delicatula* outbreak in South Korea suggest that sooty mold severity was somewhat consistent across landscapes, however, no formal assessments or quantification were conducted. Anecdotally, we, along with other *L. delicatula* researchers in Pennsylvania, have observed inconsistencies of sooty mold severities within and between land use type, calling into question the determinants of sooty mold severity. It is unclear whether severity of sooty mold is most determined by the volume of honeydew, the abiotic conditions of the substrate, the causal taxa first colonizing the honeydew, and/or other ecological variables. Moreover, where sooty mold is severe, there remains numerous open questions about the effects of sooty mold on the phyllosphere, and the direct and indirect effects of sooty mold on plant and ecosystem health and resilience.

Despite these open questions, this work is the first to attempt to characterize the identity of sooty mold taxa associated with this invasive insect. Because to date no lures or attractants are available for use in trapping *L. delicatula*, monitoring and detecting populations of this insect are extremely challenging, particularly when the insects are in low abundance (e.g., when just having made a jump or incursion into a new, disjunct

region). The spread of established populations of *L. delicatula* in the eastern US to date have generally followed transportation corridors, particularly rail lines and highways. Being highly disturbed, these corridors harbor high abundances of *A. altissima*. Therefore, identification of key or “indicator” *L. delicatula*-associated sooty mold taxa on *A. altissima* (first by molecular identification, and subsequently by morphological examination) has the potential to serve as a proxy or “finger-print” of this invasive insect that could greatly facilitate improved detection of this pest and help to limit its further spread.

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Chapter 3

High-throughput sequencing of phyllospheric fungal communities on *Vitis riparia* and *Vitis vinifera* over 5 weeks of exposure to *Lycorma delicatula* and associated sooty mold.

Lycorma delicatula, or Spotted lanternfly (Hemiptera: Auchenorrhyncha), is an invasive phloem-feeding insect pest of forest and agricultural systems. The sugary waste of phloem-feeders, honeydew, indirectly affects plant health by disturbing microbial communities on the surfaces of above-ground plant tissues, or the phyllosphere. Honeydew supports the development of sooty mold, a polyphyletic group of fungi that can coat affected plants in dark mycelial mats and diminish the plant's capacity for photosynthesis, heat and drought tolerance, and defense. Little is known about the effects of honeydew from *L. delicatula* on phyllospheric communities, or about the identity of sooty mold fungi in this system. A previous study showed that fungal diversity decreased over time, and that communities became more similar to one another, indicating that honeydew influenced the fungal community structure after *Ailanthus altissima* leaves were exposed to *L. delicatula*. We also found evidence that a species of *Trichomerium* was primarily responsible for sooty mold. In the present study, we expanded our investigation to determine whether these trends would be similar on other plant host species. We used high-throughput amplicon sequencing of the ITS1 gene region to characterize fungal communities on leaves of *Vitis vinifera* ('Chardonnay') and *Vitis riparia* (river grape), two preferred hosts of *L. delicatula* over several weeks of exposure to *L. delicatula* on a residential property in Berks County, Pennsylvania in 2017. We found that fungal community composition differs between the two *Vitis* species with fungal communities on *Vitis riparia* being less diverse than those on *Vitis vinifera*,

though diversity slightly decreased over exposure to *L. delicatula* in both. Finally, we found two genera of sooty mold, *Trichomerium*, which was significantly more abundant in *V. riparia*, and *Cladosporium*, which was nominally more abundant in *V. vinifera*. Our findings in this and a previous study indicate that while sooty mold from *L. delicatula* may consist of a mix of fungal taxa, *Trichomerium* may have some stronger association with *L. delicatula* than do other sooty mold fungi.

Introduction

Spotted lanternfly, or *Lycorma delicatula* (Hemiptera: Auchenorrhyncha), was first detected in the United States in 2014 (Barringer et al. 2015). *L. delicatula* is a large-bodied, voracious phloem-feeder with over 70 known woody-stemmed plant hosts in the northeast United States, including wild and cultivated *Vitis* species (Vitales: Vitaceae) (Barringer & Ciafré, 2020). Direct damage from feeding by *L. delicatula* includes loss of vigor, wilting, and reduced overwintering success. However, indirect damage from *L. delicatula* can be as critical as direct damage. *L. delicatula* consumes large volumes of phloem sap, and excretes correspondingly large volumes of its sugary waste, honeydew (Urban et al. 2021). Honeydew accumulates on any surfaces beneath actively feeding *L. delicatula*, including the leaf surfaces of host plants (Barringer & Ciafré, 2020). Studies on other sap-feeding insects have shown that large accumulations of honeydew on leaves affect carbon-cycling (Santos et al., 2013), manipulate plant defenses (Schwartzberg & Tumlinson, 2014), and alter the microbial communities found on the surface of above-ground plant tissues, or the phyllosphere (Menkis et al. 2015).

Trophic interactions such as those found between plants, phloem-feeders, and the sooty molds facultatively associated with them can change phyllospheric community assemblages (Perez et al. 2009). Phyllospheric communities are comprised of diverse microbial species (e.g., bacteria, archaea, fungi, viruses, algae, etc.) engaged in complex and often highly dynamic inter- and intra-species processes, that to date are not well understood, but known to potentially impact plant health (Rastogi et al. 2013). Phyllospheric communities play a role in regulation of primary and secondary plant metabolism, and changes to those communities have been shown to affect water uptake, and pathogen and herbivore resistance. (Rosado & Almeida 2020; Smets & Koskella 2020; Stone et al. 2018).

In systems such as *L. delicatula* infestations, in which phloem-feeders can feed and deposit honeydew on plant hosts over many weeks, honeydew disturbs the phyllosphere, and filters the community members to those that can thrive in the environment it creates. On the leaf surface, epiphytes must already be adapted to an environment with high UV exposure (Carvalho & Castillo 2018; Koskella 2020). The presence of honeydew poses another hurdle for microbes, low water availability due to its high sugar content and high osmotic pressure (Douglas 2006). Honeydew is a carbohydrate-rich resource but varies broadly in its composite saccharides, including oligo- and poly- saccharides (Wilkinson et al. 1997). Because not all sugars can be digested by all microbes, the composition of sugars on a leaf surface can regulate phyllospheric communities (Perazzolli et al. 2020). Honeydew is also a resource-limited in amino acids and micronutrients, though the availability of each varies greatly, especially when compared between the species of phloem-feeders producing honeydew

and their plant hosts (Pringle et al., 2014). This nutritional variation further limits the microbial community members which can thrive on a honeydew-coated leaf surface.

Honeydew facilitates the growth of sooty mold, a polyphyletic group of fungi which are facultatively associated with phloem-feeders and their waste (Schmidt et al. 2014). Though sooty molds are not known to be direct plant pathogens, mild and severe infestations of sooty mold can harm plant health. In mild infestations, even before sooty mold is visible to the naked eye, the fungi out-compete existing microbes and dominate phyllospheric communities exposed to honeydew (Menkis et al. 2015). In severe infestations of sooty mold, the fungi form thick black mycelial mats that cover leaf surfaces and stress plants by reducing their photosynthetic capacity, increasing heat stress, and preventing normal transpiration (Chomnunti et al. 2014). Black mold on ornamental plants and cultivated fruit can lower their economic value (Urban et al. 2021).

Severe outbreaks of sooty mold have been observed in association with *L. delicatula* in the northeast United States, especially on ornamental plants and in forest systems. The long-term impacts of sooty mold on key members of ecosystems, even those which are not hosts of *L. delicatula* but lie beneath them, are not known. Damage to wine and table grapes (*Vitis*) have represented a large portion of the economic impacts of *L. delicatula* since its arrival in the United States (Urban et al., 2021). Despite this, relatively little sooty mold development has been observed on cultivated grapes, even in vineyards not treated with fungicides (Urban et al., 2019). This is particularly surprising because when *L. delicatula* became invasive in Korea in 2008, sooty mold was the most economically devastating mode of damage caused by *L. delicatula* in cultivated grapes (Song, 2010). The reasons for the observed differences in these systems is not yet known.

In fact, little is understood about the factors determining the severity or identity of sooty molds in any system. Sooty mold colonies are comprised of mixed fungal taxa which can vary across samples even given the same substrates, insect species, and plant hosts (Hughes & Seifert 2012). It has been generally accepted that there is no relationship between the plant-insect nexus and specific sooty mold fungi. However, macro- and micro- nutrient contents in honeydew of other sap-feeders vary by plant host and phloem-feeding species where the conditions on honeydew-coated leaves would select for specific sooty mold fungi (Hendrix et al 1992; Pringle et al 2014). It can therefore be expected that certain sooty mold fungi would be successful given honeydew is deposited by a particular phloem-feeding insect on a particular plant host species (O'Malley 2008).

A clearer understanding of sooty mold communities has been complicated by limitations associated with identification of fungi, that only recently have been improved. Sooty molds are often difficult to isolate, and morphological descriptions of mixed colonies can be complicated by the prevalence of pleomorphy amongst sooty mold fungi (i.e., the ability of most fungi to shift between sexual and asexual states, sometimes even multiple asexual states, and with it, their appearances, in response to different environmental conditions). Use of DNA sequencing for fungal identification is a promising solution, however until recently, amplicon sequencing of fungi has been limited because bioinformatic pipelines were largely designed with bacteria as their model Kingdom, which was a poor fit for the evolutionary history of fungi and because reference databases for fungi have been sparse and complicated by dual nomenclature (Müller & Ruppel 2014). Emergent methods in amplicon sequencing, from amplification to bioinformatics, along with increased resolution of dual nomenclature, have

revolutionized perspectives on fungal ecology broadly (Hibbett & Taylor 2013); there is reason to believe that further applications of these methods could better inform sooty mold research.

It is not known whether the specific nature of the changes to phyllospheric fungal assemblages brought on by exposure to *L. delicatula* are most related to the insect as a facilitator and the composition of its honeydew, the colonization and succession patterns as controlled by the local gamma diversity of fungi and the initial phyllospheric communities, or the life histories and/or historical geographies of all actors in the system. *L. delicatula* continues to spread in the U.S. As it establishes in new areas and habitats, the ecological contexts determining the effect of sooty mold will change, so it is important that researchers do not underestimate potential risk from sooty mold in any agricultural and forest systems (D. -H. Lee et al., 2019). A better understanding of sooty mold is and will be critical to mitigating harm by *L. delicatula*. The first step must be to identify potential causal taxa of sooty mold found in association with *L. delicatula*.

In a previous experiment using amplicon sequencing (Taleb et al 2022, in review), we found that on the leaves of *Ailanthus altissima* affected by *L. delicatula*, fungal assemblies become richer, less even, and more similar to one another over a season of exposure. We also found the abundance of *Trichomerium* was correlated with observations of sooty mold symptoms. The study was limited, however, in that it was restricted to a single plant host.

In this study, we continued to assess fungal communities disturbed by the honeydew of *L. delicatula* on the leaves of *Vitis vinifera* and *Vitis riparia*. *Vitis* species are a strong model to investigate these patterns in relation to *L. delicatula*, because so

much is known about the microbial communities found in the phyllosphere of both wild and cultivated *Vitis*, and the variables that shape those communities. *V. vinifera* and *V. riparia* specifically pose an opportunity because each is situated in a key landscape of concern and are highly attractive hosts for *L. delicatula* (Barringer & Ciafré 2020). We expected communities to become more similar to one another over time, even between plant host species, and less diverse. We also expected to find *Trichomerium* abundance to rise with time, indicating an association with the development of sooty mold.

Methods

Sampling

Leaf surface communities were sampled at a residential property with no known history of pesticide use in Berks Co., PA (40.40°N, 75.71°W) over approximately nine weeks from August 3 - October 4th, 2017. Sampling dates were between six and nine days apart (Table 3-1). The sampled property is located approximately four miles from the site at which *L. delicatula* was first detected in Pennsylvania (Barringer et al. 2015). Five cultivated grape vines (*Vitis vinifera*, “Chardonnay”) were selected from a row of untreated vines on the property, and five river grape vines (*Vitis riparia*) were selected from along the adjacent forested path on the property. Secondary or tertiary branches from vines were selected and marked with hanging tags attached with fishing wire that was sealed with crimping beads. The tagging method was selected to minimize interference with *L. delicatula* by visual or chemical stimuli. For each plant, the same frond was sampled throughout the study. In some instances, vines or selected fronds

wilted or died from feeding damage by *L. delicatula*. In these instances, no samples were taken from that individual for any weeks following death. Fungal communities were sampled with BD CultureSwab™ (BD Diagnostics, Franklin Heights NJ) sterile swabs. For each sample, tips were swabbed across the adaxial surfaces of one leaf for 30 seconds. Care was taken to swab across the entire surface of each leaf, and to use enough pressure to dislodge epiphytic cells without visibly damaging leaf surfaces, then stored in the collection tube included with the swabs in chilled coolers while they were transported back to the lab, where they were stored at -20° C until DNA. At the time of collection, continued presence of *L. delicatula* was confirmed for each vine, and observations of the presence of visible sooty mold development were recorded for each branch.

DNA extraction, amplification, and sequencing

DNA was extracted from swabs using the DNeasy PowerSoil Kit (QIAGEN USA, Germantown, MD), according to the manufacturer's protocols for samples with potential polymerase chain reaction (PCR) inhibitors, including leaf surfaces. For each extracted DNA sample and for three water controls, the ITS1 gene region was amplified in triplicate by PCR with primers ITS1f-ITS2 (EMP.ITSkabir; Thompson et al. 2017) using the Platinum Hot Start 2X Master Mix kit (ThermoFischer Scientific, Santa Clara, CA) according to the Earth Microbiome Project protocols for ITS (Thompson et al., 2017). Sample-specific barcodes were then attached to PCR products, and triplicate barcoded PCR products were then pooled by sample. Pooled PCR products were purified using ExoSAP-IT™ (ThermoFischer Scientific, Santa Clara, CA). Pooled and purified samples

and controls were then sent to the Genome Sciences Facility at the Penn State College of Medicine (Hershey, PA) for sequencing on the Illumina MiSeq platform.

Data processing

The platform ‘Quantitative insights into microbial ecology 2’ (QIIME2 version 2018.8; <https://qiime2.org/>; Bolyen et al., 2018) was used to trim, merge, filter, denoise, and assign taxonomy to amplicon sequence reads from a dataset that included the samples analyzed in this study. Adapters and conserved regions were trimmed from unmerged sequences using the ITSxpress plugin, using settings for ITS1 gene region, and fungal taxa only (Rivers et al. 2018). The q2-dada2 plugin was used to quality filter, denoise and pair sequence reads, and to remove chimeras, using default settings (Abarenkov et al., 2021). In this step, forward and reverse reads were truncated for quality to 190 and 220 bp, respectively. The q2-dada2 plugin finally clustered paired and filtered reads into exact amplicon sequence variants (ASVs).

To assign taxonomy, a naïve Bayesian classifier was trained on the UNITE version 10.05.2021 dynamic database (Nilsson et al. 2019) and the QIIME2 feature-classifier plugin was used to assign taxonomy to ASVs. The ASV and taxonomy table were then exported as .biom files for further analysis. Scripts and data necessary to reproduce data processing are available on GitHub (<https://github.com/mariam-taleb/Qiime2-Unite-ITSphyllosphere>).

Data analysis

To test our hypotheses about the community assembly as represented by exact amplicon sequence variants (ASVs), data analyses were conducted in R (R Core Team, 2017), and visualized with the `ggplot2` package (Wickham, 2016). The `phyloseq` package was used to extract and manipulate data sets created in QIIME2 bioinformatics processing (McMurdie & Holmes, 2013). Because data processing was done with for all samples that had been sequenced along this those in this study, including samples for other experiments, the data were subset to only samples for this study, and only those in weeks 3, 4, 5, 7, and 8--the sampling weeks for which there were sufficient replicates. The data were then normalized by a centered log-ratio (clr) transformation using the `ALDEx2` package (Fernandes et al., 2014; Galloway-Pena et al., 2017). ASV accumulation curves were generated using the `vegan` package (Oksanen et al., 2020) to estimate sampling depth.

To estimate alpha diversity, Shannon's entropy and Gini-Simpson diversity indices were calculated, which give greater weight to evenness and richness respectively, using the `hillR` package (Li, 2018). To test whether there was an effect of *Vitis* species and collection date on richness or evenness of the fungal community, mixed effect models were fit to each of the diversity indices, with plant species and collection date as fixed effects, and individual sampled plant as a random effect, using the `lme4` and `lmerTest` packages (Bates et al., 2020; Kuznetsova et al., 2017). Estimated marginal means for plant species and collection date were computed, as were contrasts among them to assess the direction of those effects using the `emmeans` package (Lenth 2022).

A Bray-Curtis dissimilarity matrix was calculated using the phyloseq package to estimate differences between fungal assemblages in all samples. Non-metric multidimensional scaling (NMDS) was used to visualize pairwise dissimilarities using the ordinate function of the phyloseq package (Cordier et al., 2012; Humphrey et al., 2014). Dispersals (variance) around the medians of samples by week and plant species were calculated using the betadisper function in the vegan package, then the permutest test function was used to test for homogeneity of dispersals. To test for an effect of *Vitis* species and collection date on fungal assemblages, the adonis function of the vegan package was then used to perform a Permutational Analysis of Variance (PerMANOVA).

Relative abundances of taxa were calculated from untransformed sequence counts, divided by the total number of sequence reads from all samples. Relative abundances of all fungal orders in *Vitis riparia* and *Vitis vinifera* were visualized with ggplot2. The topmost abundant genera, representing >2% of all sequences reads, are highlighted in the plot. 2% of sequences reads was selected as the cutoff because below 2%, total abundances drop off precipitously. Differential abundances were calculated using the DESeq function from the DeSeq2 package (Love et al. 2014). Untransformed counts were used in this analysis because the package performs its own normalization.

All scripts and data required to reproduce analyses are available on GitHub (https://github.com/mariam-taleb/ITS_Vitis-SLF_Ranalysis).

Results

Bioinformatics

After initial data processing, samples with fewer than 1000 reads were pruned, of which there was only one—*Vitis vinifera*, plant 12, sampled in week 4. We struggled to successfully amplify the gene region from several samples due to an error in PCR protocols which we later corrected. As a result, samples from weeks 1, 2, 6, and 9 of our nine-week study (Table 3-1) contained only one replicate of either *Vitis vinifera* or *Vitis riparia* and were therefore excluded from the analysis. In the remaining dataset, there were 28 total samples, containing 2,173,984 total sequence reads, which represented 3277 taxa. The data had 99.68% sparsity, and 8.54% of all ASVs (i.e., amplicon sequence variants) (15019 reads) were singletons.

Table 3-1: Total number of sequence reads (sequencing depth) for each individual plant for each week it was sampled. Sampling dates are all in 2017.

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
	3-Aug ²	9-Aug	18-Aug ³	24-Aug	31-Aug	8-Sep	14-Sep	25-Sep	4-Oct
VV^{1a} -12				53 ⁴	7996	8979	11564	9949	6991
VV -13			4641		13889		2443	7578	4987
VV -14			11182	6762	4754	9459	13846	7276	3355
VV -15				7092	11858	5360	7098	11383	6898
VV -16		6918			8827	14284	4137		7985
VR^{1a} -17			7248	5901	5628	4660	1917	9545	
VR -18			8847	4395	6259		7762	7621	9212
^{1a-b} VV: <i>Vitis vinifera</i> ; VR: <i>Vitis riparia</i> . ² Swabs were not collected of <i>Vitis</i> samples in this week. ³ PCR protocols were corrected in this week. ⁴ Sample was removed from analyses due to low sequencing depths.									

ASV accumulation curves were constructed for each sample after normalization (Fig. 3-1). All samples approached their respective asymptotes, indicating sufficient sampling depth for all samples. Accumulation curves also indicated that fewer ASVs were detected in samples from *Vitis vinifera* than those from *Vitis riparia*. Because all samples approached asymptotes, it can be assumed that a true difference is being observed, rather than an effect of some difference in inhibitors to extraction and/or amplification affecting sequencing.

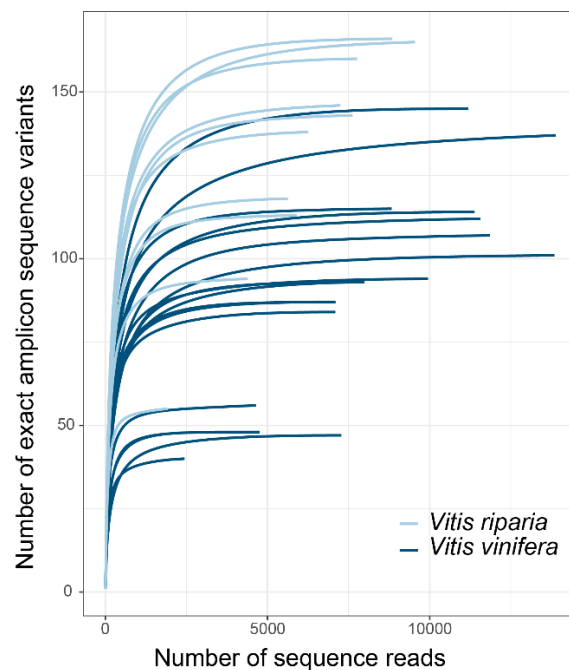


Figure 3-1. Accumulation (rarefaction) curves showing relationship between total number of sequence reads obtained in each fungal community sample and the number of exact amplicon sequence variants (which serve as a proxy for ‘species’) observed in each fungal community sample.

Alpha diversity

Shannon’s entropy indices ranged from 188.5 to 519.4 with a mean of 258.2 in *Vitis riparia* samples and ranged from 39.36 to 275.51 with a mean of 122.08 in *Vitis*

vinifera samples. A mixed-effect model was fit to Shannon indices by plant species, with week as an interaction effect, and individual plant as a random effect. Simplifying the model did not reduce AIC. Residuals indicated there may be an outlier, so we conducted a Student's test with Bonferroni correction, which found that *Vitis riparia* #17, sampled in week 7, was an outlier ($p=0.01373$). The sample was removed from the model because no ecological explanation could be found, but a low number of ASVs in that sample indicated potential sampling errors. The model found that plant species had a significant effect on Shannon diversity ($p < 0.001$), but collection week was not significant, nor was there an interaction effect (Table 3-2). We also found a non-significant downward trend in Shannon diversity over collection dates (Fig. 3-2a). We then calculated the estimated marginal means of Shannon diversity indices for both plant species at each week and found that Shannon diversity was higher in *Vitis riparia* than in *Vitis vinifera*, and significantly so weeks 4, 5, and 7 (Fig. 3-2b).

Table 3-2: Mixed Effect Model results for Shannon diversity indices versus plant species, collection week, and an interaction effect of plant species and collection week.

Mixed Effect Model Results			
(Shannon diversity~ plant species * collection week)			
Predictors	Estimates	CI	p
(Intercept)	272.14	183.82 – 360.46	<0.001
Plant species [<i>V. vinifera</i>]	-119.63	-236.19 – -3.06	0.045
Collection week	-8.27	-23.72 – 7.19	0.279
Plant species [<i>V. vinifera</i>] * Collection week	3.29	-16.38 – 22.96	0.731
Random Effects			
σ^2	1736.84		
τ_{00} plant	214.73		
ICC	0.11		
N_{plant}	7		
Observations	27		
Marginal R² / Conditional R²	0.582 / 0.628		

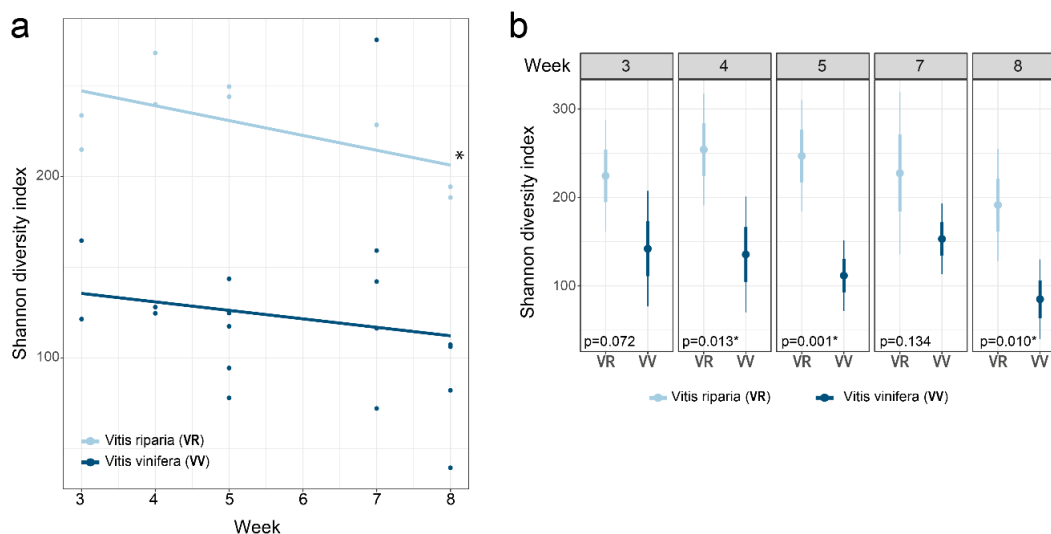


Figure 3-2. Shannon diversity indices for fungal communities taken from *Vitis riparia* and *Vitis vinifera* over time. **a)** Line plot showing Shannon diversity over time. Each fungal community is plotted as a point. The slopes of Shannon diversity indices for each plant species are plotted as lines; slopes calculated by *lm*. * $p < 0.05$ (Table 3-2.) **b)** Box plots showing estimated marginal means of Shannon diversity indices for fungal communities sampled from *Vitis riparia* and *Vitis vinifera* at each week.

Gini-Simpson indices ranged from 52.06 to 75.91 with a mean of 64.80 in *Vitis riparia* samples and ranged from 4.94 to 39.74 with a mean of 26.83 in *Vitis vinifera* samples. A mixed-effect model was fit to Gini-Simpson indices by plant species, with week as an interaction effect, and individual plant as a random effect. Simplifying the model did not reduce AIC. Plant species had a significant effect on Gini-Simpson diversity ($p < 0.001$), but the model found no significant main or interaction effect of collection week (Table 3-3). As with Shannon diversity, we found a non-significant downward trend in Gini-Simpson diversity over collection dates (Fig. 3-3a). The estimated marginal means of Gini-Simpson diversity indices for both plant species at each week indicated that for all weeks, Gini-Simpson diversity was significantly higher in *Vitis riparia* than in *Vitis vinifera* (Fig. 3-3b).

Table 3-3. Mixed Effect Model results for Gini-Simpson diversity indices versus plant species, collection week, and an interaction effect of plant species and collection week.

Mixed Effect Model (Gini-Simpson~ plant species * week)			
Predictors	Estimates	CI	p
(Intercept)	76.47	61.31 – 91.64	0.001*
plantsp [VV]	-38.90	-58.95 – -18.84	0.001*
week	-2.16	-4.64 – 0.31	0.084
plantsp [VV] * week	0.44	-2.78 – 3.65	0.780
Random Effects			
σ^2	49.01		
τ_{00} plant	14.07		
ICC	0.22		
N _{plant}	7		
Observations	28		
Marginal R ² / Conditional R ²	0.845 / 0.880		

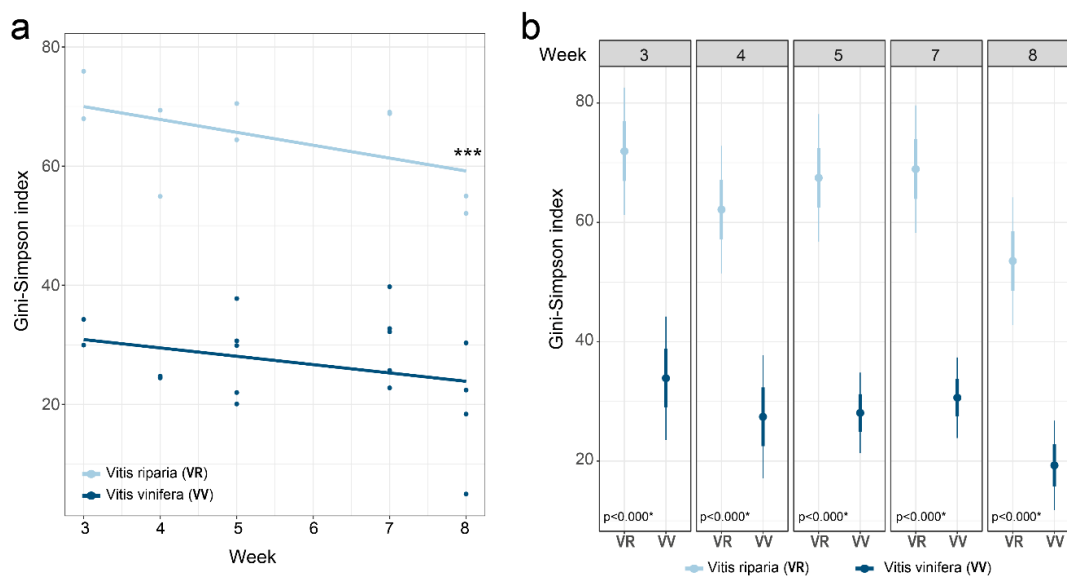


Figure 3-3. Gini-simpson diversity indices for fungal communities taken from *Vitis riparia* and *Vitis vinifera* over time. **a)** Line plot showing gini-simpson diversity over time. Each fungal community is plotted as a point. The slope of gini-simpson diversity indices for each plant species plotted as lines; slopes calculated by *lm*. *** $p \leq 0.001$ (Table 3-3.) **b)** Box plots showing estimated marginal means of gini-simpson diversity indices for fungal communities sampled from *Vitis riparia* and *Vitis vinifera* at each week.

Beta diversity

The dissimilarity between fungal assemblages was estimated by Bray-Curtis matrix (Fig. 3-4). The results of betadisper test, indicated that dispersals did not significantly vary between plant species ($p=0.346$), between weeks ($p=0.480$), or between individual plants ($p=0.486$). A PerMANOVA was used to model the effect of plant species, collection week, and an interaction effect. We found a significant effect of plant species on fungal assemblages, but no effect of collection week or an interaction of plant species and collection week (Table 3-4).

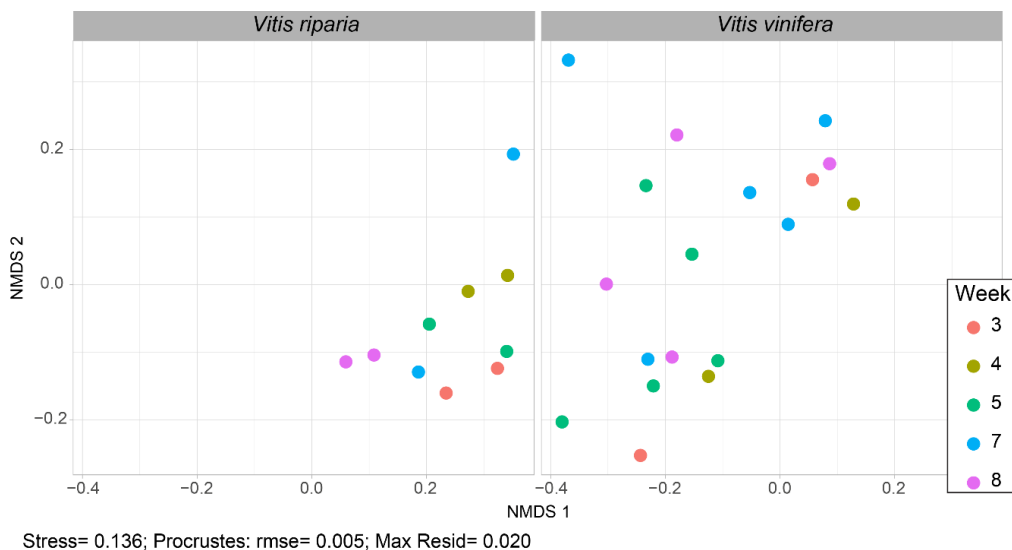


Figure 3-4. Non-Metric multidimensional scaling of Bray-Curtis dissimilarity indices plotted by sample. *Vitis riparia* samples plotted in left panel, *Vitis vinifera* samples plotted in right panel. Each sample point colored by week of collection.

Table 3-4. PermANOVA results for fungal community dissimilarity (Bray-Curtis) by collection week, plant species, and an interaction of collection week and plant species.

	Df	Sums of Sqs	Mean Sqs	F statistic	R ²	Pr(>F)
Collection Week	4	0.854672	0.213668	0.9654347	0.1362227	0.594
Plant Species	1	0.7280901	0.7280901	3.289793	0.1160474	0.001
Week : Plant species	4	0.7075923	0.1768981	0.7992939	0.1127803	0.979
Residuals	18	3.9837226	0.2213179	NA	0.6349496	NA
Total	27	6.274077	NA	NA	1	NA

Relative abundances

Of 217,398 total sequence reads, we were unable to identify 29.38% to genus (63876 reads), 13.17% to family (28640 reads), 10.40% to order (22625 reads), 10.14% to class (22055), and 6.68% to phylum (14526). Ascomycota made up 74.44% of sequences (161850 reads), while Basidiomycota made up only 18.87% of sequences (41022 reads). Sequence reads were dominated by a few classes. Dothideomycetes made up 58.46% of total reads (127101 reads), while its sister clade, Eurotiomycetes, made up another 7.92% (17221 reads). Tremellomycetes made up 10.97% of total sequences (23867 reads)— which represented 58.18% of all sequences within Basidiomycota. Similarly, at the genus level, sequence reads were dominated by a few genera (Fig. 3-5). Two of those genera contain known sooty molds--*Cladosporium*, and *Trichomerium*. Differential abundance analyses indicated that *Cladosporium* was more prevalent in *Vitis vinifera*, though not significantly so, and *Trichomerium* was significantly more abundant in *Vitis riparia* (Table 3-5).

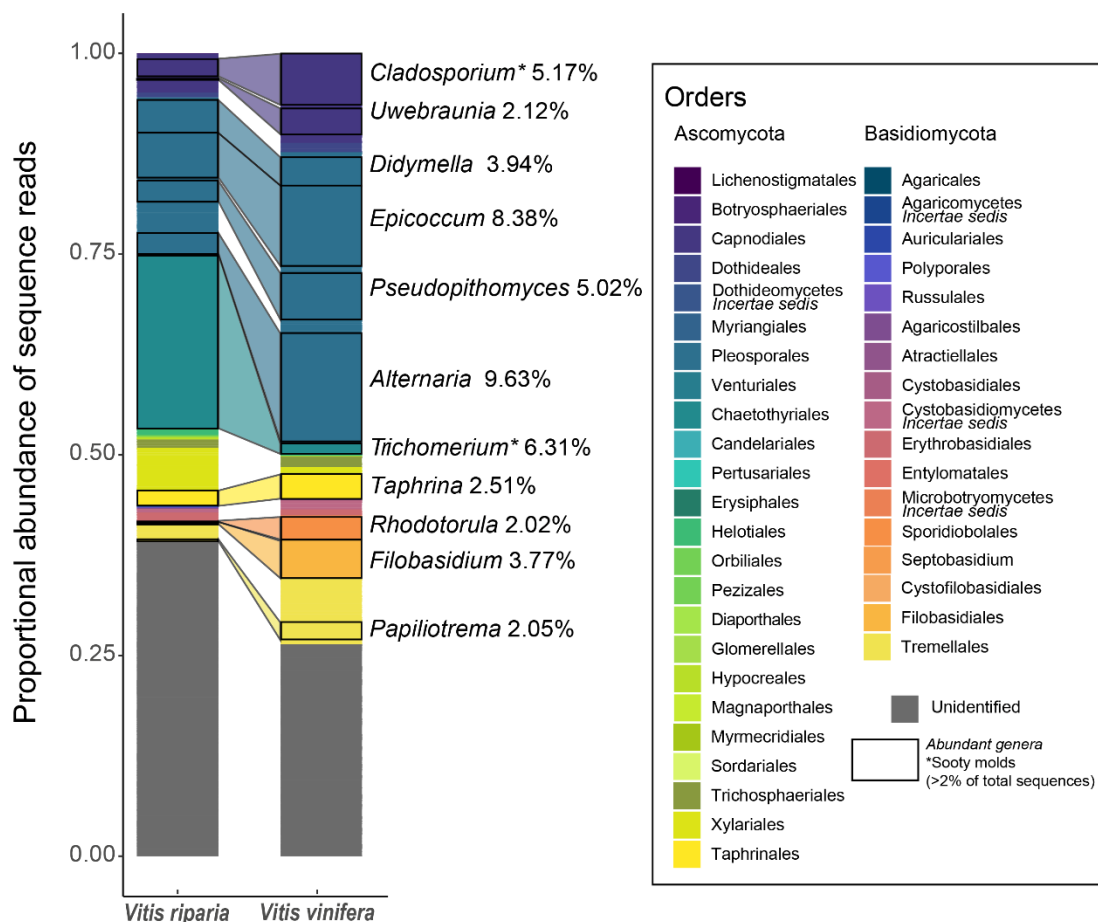


Figure 3-5. Relative proportional abundance of taxa, colored by order. Most abundant genera within order outlined in black. Differences between *Vitis* species for most abundant genera highlighted between bars, percentages note the total abundance of each genus for all samples.

Table 3-5. Log₂ fold change of normalized counts from *Vitis vinifera* to *Vitis riparia*. For positive values, the mean abundance in *Vitis riparia* is greater. Table shows only fungal genera which significantly differ in abundance between *Vitis* species.

	Base Mean ¹	Log ₂ Fold Change	Standard Error	Wald statistic	p-value	p-adj
Alatosessilispora	42.73117	9.799687	1.913466	5.121434	0.0000	0.0000*
Rhodotorula²	62.73803	-8.20071	1.583871	-5.17764	0.0000	0.0000*
Devriesia	67.77569	9.915555	2.112524	4.6937	0.0000	0.0001*
Trichomerium²	1095.37	4.805784	1.086127	4.4247	0.0000	0.0003*
Wojnowiciella	41.79228	7.241265	1.692448	4.278575	0.0000	0.0006*
Arthrocatena	38.66385	5.977681	1.414116	4.227149	0.0000	0.0006*
Aureobasidium	58.68533	-8.09784	2.12376	-3.81297	0.0001	0.0031*
Strelitziana	56.21975	5.059879	1.443301	3.505767	0.0004	0.0090*

¹ Mean of normalized counts taken over all samples. ² Abundant genera (<2% of all sequence reads).

Discussion

We compared the phyllospheric fungal communities on two *Vitis* species over a season of exposure to *L. delicatula* and explored causal sooty mold fungi on both *Vitis vinifera* and *Vitis riparia*. Plant species influenced the effect of exposure to *L. delicatula* honeydew on the phyllospheric fungal community. Relative abundance analyses also indicated *Trichomerium* was a dominant causal sooty mold fungus on *Vitis riparia*, and *Cladosporium* was a prominent causal sooty mold fungus on *Vitis vinifera*.

Fungal communities on *Vitis riparia* were richer and more even than those on *Vitis vinifera*, which mirrored our observations from rarefaction curves, in which more ASVs were detected in *V. riparia*. We also find that total assemblages of fungal communities differ between plant species. *V. riparia* assemblages were more similar to one another than were *V. vinifera*. Initial community assemblages shape which sooty mold taxa would subsequently successfully colonize honeydew coated leaf surfaces. Richness and evenness decrease over weeks of exposure to *L. delicatula*, but not significantly so. This indicates that the pressure of honeydew exposure restricts what taxa can be successful, and that the few that can dominate the community over time. In this experiment, we did not see a strong effect of time on alpha or beta diversity, and therefore can draw no conclusions about successional patterns. It is possible that an effect of time was obscured due to insufficient sampling, and minimal development of sooty mold in the study location.

Two of the most abundant genera, *Cladosporium* and *Trichomerium*, contain known sooty molds. *Trichomerium* was significantly more abundant in *Vitis riparia*, and *Cladosporium* somewhat more abundant in *Vitis vinifera*. This is particularly interesting because in a previous study examining fungal communities of *Ailanthus altissima* exposed to *L. delicatula*, *Trichomerium* was also identified as a potential causal agent of sooty mold, while no *Cladosporium*, or even sooty mold fungi from any family outside that of *Trichomerium* was found. To the author's knowledge, *Trichomerium* species of sooty molds have only been isolated from East Asia, especially from within the native range of *L. delicatula*, Thailand in particular, (Chomnunti et al. 2012; Hongsanan et al. 2016).

Sooty mold has previously been studied primarily in agricultural (cultivated) systems (Filho & Paiva 2006). In these studies, *Cladosporium*, and its sooty mold relatives in the order Capnodiales are very commonly found. It's been suggested that plant breeding, both traditional and contemporary can and has manipulated phyllospheric microbial communities (Rastogi et al. 2013). The difference between sooty mold found on wild-type and cultivated plants exposed to *L. delicatula* deserves further investigation. Future work should not only repeat this experiment but expand sampling to include broader ranges of sooty mold severities, plant species, and geographic locations. *In vivo* sooty mold samples should be collected and examined to bolster molecular data with morphological findings.

Amplicon sequencing of the phyllosphere of *Vitis riparia* and *Vitis vinifera* under the pressure of exposure to honeydew of *L. delicatula* showed that the whole community is

affected by honeydew as a press disturbance. Also, *Cladosporium* is a potential causal taxon of sooty mold on *V. vinifera*, and *Trichomerium* is a potential causal taxon of sooty mold on *V. riparia*. Though our results cannot confirm a singular causal agent in *L. delicatula*-associated sooty mold, they do indicate that there may be at least one taxon commonly found in association with *L. delicatula*, but not commonly found in this geographic region otherwise. A great deal of research has treated sooty mold as a pseudo-pathogen in that it has followed the logic of Koch's postulates to prove causality of a single microbe with a consistent symptomology (Chomnunti et al 2014; Hughes & Seifert 2012). For the purposes of combatting sooty mold and its insect pest facilitators, though, it may be helpful to use looser expectations to define systems—rather than looking for single causal agent, we suggest seeking sooty mold species which are common in a given system, and not common in others.

In this case, identification of the *Trichomerium* species in this system and establish its (in)consistency across the system may help to inform a monitoring method for *L. delicatula* that does not require finding the insect itself. This could be especially crucial because, so far, *L. delicatula* have primarily been detected once new populations are thoroughly established. Continued molecular and morphological work should be conducted to establish whether this is a viable path to better management.

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Chapter 4

Ultrastructure of epiphytic sooty mold on plants colonized by Spotted lanternfly (*Lycorma delicatula*: Hemiptera) in Pennsylvania (United States)

Sooty mold, a dark fungal mat which grows on leaf surfaces exposed to honeydew, plays a major role in the damage that Spotted lanternfly (*Lycorma delicatula*: Hemiptera) causes in forest, ornamental, and agricultural landscapes. However, the causal fungus (or fungi) of Spotted lanternfly-related sooty mold is not known, and in the United States the range of severity of sooty mold has been broad and unpredictable. To determine the causal fungi of sooty mold, and to investigate whether the composition of those fungi is consistent across Spotted lanternfly-affected plants, or is correlated with plant species, sooty mold symptomology, or geographic range, we collected weekly samples of sooty mold from multiple plant species across Pennsylvania during the adult stage of the insect. We then examined the colony morphology and ultrastructure of samples by light microscopy and scanning electron microscopy to characterize the composition of sooty mold in these samples, and to explore the variables that determine it. Though the fungal colonies of Spotted lanternfly sooty molds were heterogeneous, a morphospecies of *Trichomerium* is present in all samples, and dominant in all samples with mild sooty mold. This implies that while the whole community may vary across several interactive factors, this morphospecies of *Trichomerium* is central in the formation of sooty mold symptomology in relation to Spotted lanternfly.

Introduction

Sooty mold is used to describe an ecological group of melanized epiphytic fungi from sister classes Dothideomycetes and Eurotiomycetes which grow on sugary plant exudates and the waste excretions of phloem-feeding insects and are common in humid temperate and tropical climates (Chomnunti et al. 2014). Sooty mold fungi can feed on plant exudates, but they are most often associated with phloem-feeding insects. Sooty molds feed on the sugary waste of phloem-feeding insects, called honeydew, and form darkly pigmented mycelial mats which cover surfaces where honeydew accumulates, including and most typically on the plant hosts of phloem-feeders. There is no evidence of direct parasitism of leaf tissue by sooty mold, or of any penetration of their hyphae into the leaf surface (Filho and Paiva 2006). However, mycelial mats physically obstruct as much as 77% of total incident light, restricting photosynthesis in severely affected plants (Filho and Paiva 2006; Insausti et al. 2015). Sooty molds can affect the regulation of stomata by altering the humidity and temperature of the microenvironments that stomata are exposed to, thereby altering gas exchange on affected leaves (Santos et al. 2013). Moreover, coverage by dark mycelia can trap heat at the leaf surface and directly increase heat stress (Kim, Kweon et al. 2016).

Sooty mold colonies are typically composed of admixtures of fungal taxa from of the approximately 20 genera containing fungi identified in the literature as capable of causing sooty mold (Chomnunti et al., 2014). Most sooty mold research hypothesizes that, in all cases, the fungal taxa causing sooty mold are distributed randomly throughout the environment from the global to the hyper-local scale, and randomly mixed on any given

plant fed upon by any given phloem-feeder (Hughes 1976). Under this hypothesis, fungi capable of colonizing honeydew are ubiquitous in the environment, and stochastic ecological processes bring them to freshly deposited honeydew. However, recent research as identified conditions and interactions that select for specific fungi, sooty molds included, suggesting more than purely stochastic processes are at play. Examples of factors that may select for particular fungi include the plant host, time (i.e., successional patterns in colonization), biogeography, and insect pest species (Flessa et al. 2021). Even changes in trophic interactions between macro-organisms and the plant can affect fungal phyllosphere communities (Perez et al. 2009). We therefore hypothesize that the composition of admixtures of sooty mold fungi are, at least in part, determined by the plant-insect interactions from which they stem.

Spotted lanternfly (*Lycorma delicatula*: Hemiptera) is an invasive insect which was first detected in Pennsylvania in 2014. It feeds on the phloem of more than 70 woody-stemmed plant species in the eastern United States, causing damage in residential, recreational, agricultural, and forested landscapes (Urban et al. 2020). *L. delicatula* causes direct economic damage through phloem-feeding on crops, ornamentals, and timber trees, leading to loss of vigor and diminished overwintering success. Indirect economic damage is also of great concern. Quarantines prevent the movement of nymphs and adults but inhibit trade. Moreover, *L. delicatula* will lay its eggs on any solid surface, meaning that quarantines must be upheld through all life stages, and, because almost all goods are potential substrates for the movement of egg masses, for most forms of trade. (Urban et al. 2021).

Because *L. delicatula* are generalist feeders, large bodied, voracious, and tend to congregate in large numbers on single plants, they deposit volumes of honeydew far larger than those seen in similar systems. The sooty mold which develops on that honeydew is therefore another critical form of indirect damage caused by *L. delicatula*. Sooty mold associated with *L. delicatula* further harms crop plants such as grapes and tree fruits which have been fed upon by *L. delicatula* by coating leaves, thereby diminishing overwintering success, and by discoloring fruits, diminishing their aesthetic value (Song 2010, Urban et al. 2020). Sooty mold can also develop on and discolor residential and recreational structures.

Both the novelty and polyphagy of *L. delicatula* offers a model to investigate what factors may determine the composition of sooty mold that colonizes its honeydew, especially across diverse habitats and plant host species. In two previous studies, this research team used high-throughput DNA amplicon sequencing to describe the epiphytic fungal communities on leaves disturbed by *L. delicatula* honeydew. We first examined fungal communities on the surfaces of leaves of *Ailanthus altissima*, or tree-of-heaven, over the course of a season of exposure to *L. delicatula*. We found that the total alpha diversity of the fungal community decreased as honeydew accumulated on leaf surfaces. We also found *Trichomerium*, a sooty mold genus, present in all samples, and in abundances that increased as visible sooty mold developed over time. While it could not be identified to species based on these sequence data, the high similarity of the sequences implied that the operational taxonomic units (OTUs, which serve as a proxy for species in microbiome studies such as these) identified as *Trichomerium* may have belonged to the same species (Taleb, Henderson, and Urban, *in review*).

We then examined the fungal communities on leaf surfaces of *Vitis vinifera* ('Chardonnay') and *Vitis riparia*. Again, we found exact amplicon sequence variants (ASVs, which are a more recent and precise alternative to OTUs as a proxy for species) which were identified as *Trichomerium* in all samples, though they were significantly more abundant in *Vitis riparia* than in *Vitis vinifera* (Taleb and Urban, *in preparation*). Because it was also found in *Ailanthus altissima*, we wondered if a history of cultivation (e.g., in *V. vinifera* versus "wild" *A. altissima* and *V. riparia*) affected the sooty mold taxa found in any system.

High throughput amplicon sequencing is a powerful tool for describing whole communities, and for examining relative abundance of community members. Though methods of analyzing large amplicon sequencing datasets have become much stronger in recent years, it is still difficult to identify taxa at finer taxonomic levels. Therefore, our sequence-based studies were limited to analyses at the genus level. Moreover, our previous studies were limited in their sampling breadth, both spatially, and in terms of sooty mold severity. It was therefore still unclear whether the trends we observed in sooty mold taxa would still be apparent with broader sampling.

This observational study seeks to describe the ultrastructure of *L. delicatula*-associated sooty mold and identify its constituent taxa. To do so we field-collected samples of sooty mold growing on leaves from *L. delicatula* plant hosts across Pennsylvania. Samples were examined by light microscopy and scanning electron microscopy. We then used morphological observations to classify sooty mold morphotypes, and to investigate their distributions across plant host species, biogeography, and sooty mold symptomology. We expected to see admixtures of sooty

mold taxa which were dominated by one *Trichomerium* morphospecies, especially in wild plant hosts, consistent with our DNA-sequence based results. We also expected to see variation in the admixtures of morphospecies which correlated with plant host species and symptomology. In so doing, we sought to provide novel insights into the sooty molds brought about by *L. delicatula* on plants in the agricultural and forest systems it invades.

Methods

Specimen Collection

From July 14 2021 to September 27 2021, we scouted weekly for field specimens of sooty mold from *L. delicatula* from the easternmost to westernmost established populations of the insect in Pennsylvania in 2021 (Fig. 4-1). We relied on reported observations of *L. delicatula* from research partners and local stakeholders to inform scouting locations, then expanded searches within identified locations based on landscape features that the researchers have observed to be preferred by *L. delicatula*., especially the boundaries of recreational, residential, and commercial landscapes, as this insect is known to be more highly abundant in edge habitats. Between one and ten samples were taken at each site. We attempted to sample from plants which represented the range of severities and qualitative textures of sooty mold and a range of plant species at each location. Although it was not possible to observe active *L. delicatula* feeding and honeydew excretion at the time all samples were taken (i.e., to ensure that the insect was feeding on the same plant species upon which it was also excreting honeydew), we

sought to sample so as to reduce conflation between the plant host *L. delicatula* was feeding on and the plant substrate on which honeydew landed. That is, we selected plants with little or no canopy overlap with other hosts of *L. delicatula* and noted adjacent species when that was not possible. For each selected plant the time of sample collection was recorded, as well as the presence and life stages of *L. delicatula*. We also rated the sooty mold coverage of the whole plant on a 0-5 scale adapted from Bovi et al. (2004) (Table 4-1).

Table 4-1. Rating scale for the coverage of whole plants by sooty mold - adapted from Bovi et al. (2004).

0	no sooty mold is visible
1	gray, brown, or black staining or speckling can be found on only a few leaves (<5% affected), or only old leaves affected
2	moderate coverage by staining/speckling symptoms (<75%), and/or a few leaves with hyphal mat formation (<5%)
3	all or nearly all leaves with at least some sooty mold symptoms, and at least a few leaves show hyphal mat formation
4	plants with moderate coverage by hyphal mats of sooty mold across whole plant, and/or some selective loss of vigor
5	plants with high coverage by sooty mold hyphal mats, often with symptoms of loss of vigor in whole plant



Figure 4-1. Sooty mold collection sites 2021. Blue points indicate specific sampling sites. Counties containing sampling sites are labelled with white bullseyes. In parantheses are the number of individual sites within each county.

We then selected representative leaves from each plant (between one and three leaves per plant, depending on leaf size), taking care to select samples with minimal evidence of other herbivory or damage. Before collecting leaves, we rated the severity of sooty mold on specimen leaves with another 0-5 scale, also adapted from Bovi et al. (2004) (Table 4-2). At this time, we also described the textures of visible sooty mold as primarily “bristly,” “flaky,” “fuzzy,” “smooth,” “speckly,” or “thin.”

Table 4-2. Rating scale for the severity of sooty mold on a given leaf- adapted from Bovi et al. (2004) based previous observations of Spotted lanternfly associated sooty molds.

0	no sooty mold detected
1	some gray/black staining is seen, often along leaf veins
2	sooty mold colonies have distinct edges giving samples a speckled appearance
3	continuous hyphal mats have formed, with <30% coverage of the sample
4	continuous hyphal mats cover between 30% and 60% of the samples
5	continuous hyphal mats cover >60% of the sample

Leaves were then removed from sample plants using forceps cleaned with 95% ethanol and were collected into paper bags. Leaf samples were stored in a cooler with ice packs and desiccant packets until they were transported to our lab on the same day. At the lab, samples were stored at room temperature in aerated petri dishes lined with dry filter papers for no longer than one week.

Light Microscopy

To encourage sporulation of fungi to provide morphological characters to facilitate taxon identification, filter papers in petri dishes were dampened with water 24 hours before

examination (Kim, 2016). To prepare slide, the entire adaxial surface of each specimen was observed with a stereomicroscope (SZ61; Olympus Scientific Solutions, Tokyo, Japan). Leaf segments, approximate 5mm x 5mm, were excised from leaves and softened in 5% KOH solution for 5 minutes, then washed with lab grade H₂O, and mounted on slides in a solution of 5% glycerol, 5% ethanol, 90% H₂O. At least one sooty mold colony was then peeled from the remaining leaf surface using #2 insect pins and stainless-steel dissection probes and mounted in the glycerol solution. At least one other sooty mold colony was peeled from the leaf surface and teased apart before slide-mounting in glycerol solution. All slides were sealed for storage with clear nail polish (InstaDri Nail Color in 'Clear', Sally Hansen, Morris Plains, New Jersey, U.S.). Slides were then imaged with a compound light microscope (CX33, Olympus Scientific Solutions).

Field Emission Scanning Electron Microscopy (FESEM)

After humidification by dampening filter papers, leaf segments (approximately 1cm²) were excised from a selection of specimens for fixation and imaging by field emission scanning electron microscopy imaging (FESEM) by the Penn State Microscopy Core (State College, Pennsylvania, U.S.). Leaf segments were fixed using methods modified from K. W. Kim (2017), with a 2% (v/v) glutaraldehyde and 2% (v/v) paraformaldehyde in 0.1 M sodium cacodylate buffer at room temperature for 2 hours, then washed in the buffer for ten minutes three times. Post-fixation with osmium tetroxide from Kim's (2017) protocol was omitted in our protocols. Leaf segments were then dehydrated in an ethanol series, three times for ten minutes for each concentration (25%, 50%, 70%, 85%,

95%, 100%, 100%). Dehydrated leaf segments were dried in a critical point dryer (EM CPD 300; Leica Microsystems, Heerbrugg, Switzerland), then coated in 10nm gold/palladium with a sputter coater (SCD-050; BAL-TEC, Balzers, Lichtenstein). The adaxial surface of segments were examined and imaged with a field-emission scanning microscope (Sigma VP-FESEM; Carl Zeiss, Oberkochen, Germany).

Results

Leaves with at least some sooty mold (i.e., gray or black spots visible to the naked eye) were common in all areas with *L. delicatula* infestations, and leaves without visible sooty mold were rare. Across nine collecting trips, we collected 45 total leaf samples from the eastern (n=13), central (n=4), and western (n=28) regions of *L. delicatula* infestation in Pennsylvania. Though sooty mold did appear to consistently become more common and more severe as *L. delicatula* matured, there were many instances in which the observed number of *L. delicatula* at a given site did not seem linearly related to sooty mold coverage. Sampling was relatively evenly distributed across sooty mold severity on specimens (Table 4-3). Nine specimens developed mycoparasites while being stored in the lab, which precluded further analyses. The nine samples which were lost had an average severity of 1.11, whereas the 36 samples that were preserved had an average severity of 2.48.

Table 4-3. Trends in collected samples by genus. Plants were marked cultivated if they were planted as ornamentals and could be assumed to have been bred and purchased, no genera were mixed between wild and cultivated. The number of samples collected from each genus is noted in column “n”. Common textures refer to the only sooty mold texture, or the mode(s) of sooty mold textures in samples.

Genera	Cultivation	n	Sooty mold severity*	Common Textures
<i>Acer</i>	Cultivated	2	2.5	Smooth
<i>Ailanthus</i>	Wild	12	1.5	Bristly, thin, speckly
<i>Amur</i>	Wild	1	5	Fuzzy
<i>Carya</i>	Wild	1	3	Speckly
<i>Cercis</i>	Cultivated	1	3	Smooth
<i>Cornus</i>	Cultivated	1	5	Flaky
<i>Euonymus</i>	Wild	1	4	Smooth
<i>Fraxinus</i>	Cultivated	1	2	Speckly
<i>Juglans</i>	Wild	2	3.5	Speckly
<i>Leonicera</i>	Wild	4	3.25	Smooth, bristly
<i>Liquidambar</i>	Wild	1	5	Bristly, fuzzy
<i>Morus</i>	Wild	2	2	Fuzzy
<i>Parthenococcus</i>	Wild	1	3	Smooth
<i>Rubus</i>	Wild	1	5	Smooth
<i>Vitis</i>	Wild	5	1.8	Flaky, bristly

* mean value if n > 1

12 of the 36 specimens were of *Ailanthus altissima* leaves. *A. altissima* is a preferred host of *L. delicatula*, and the plant species on which the insect can most readily be detected in infested areas. Other samples belonged to the genera *Leonicera*, or honeysuckles (n=5), *Vitis* (n=4), *Acer* (n=2), *Juglans* (n=2), *Morus* (n=2), or *Amur*, *Carya*, *Cercis*, *Cornus*, *Euonymus*, *Fraxinus*, *Parthenocissus*, or *Rubus* (n=1 for each). These genera represented a wide array of dicots (Fig. 4-2). We began collecting specimens early in the season, before sooty mold was commonly visible to the naked eye. For these samples, their texture was not described. Of the remaining samples, 5 were described as “bristly”, 6 as “flaky”, 3 as “fuzzy”, 8 as “smooth”, 5 as “speckly”, and 2 as “thin” (Fig. 4-3).

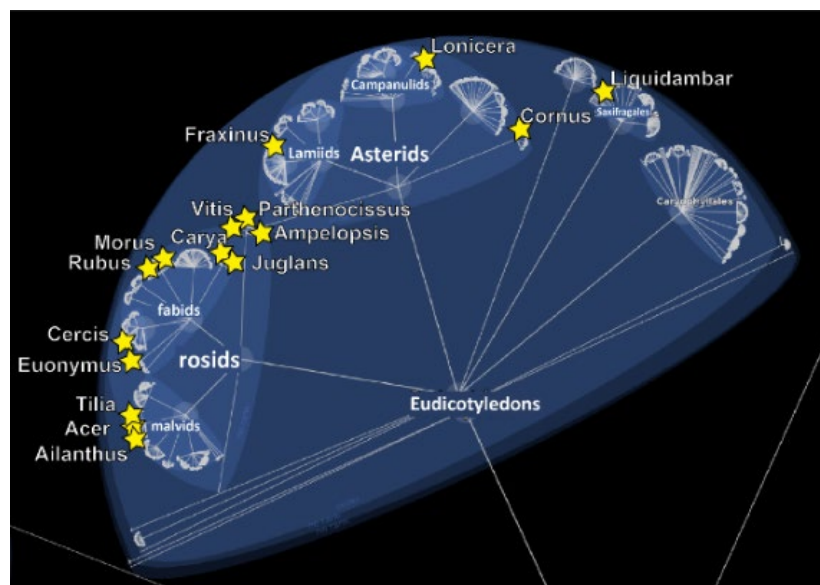


Figure 4-2: Unrooted phylogeny of Eudicotyledons, with yellow stars marking the genera from which samples were collected. Samples were spread across the majority of the diversity of dicots.

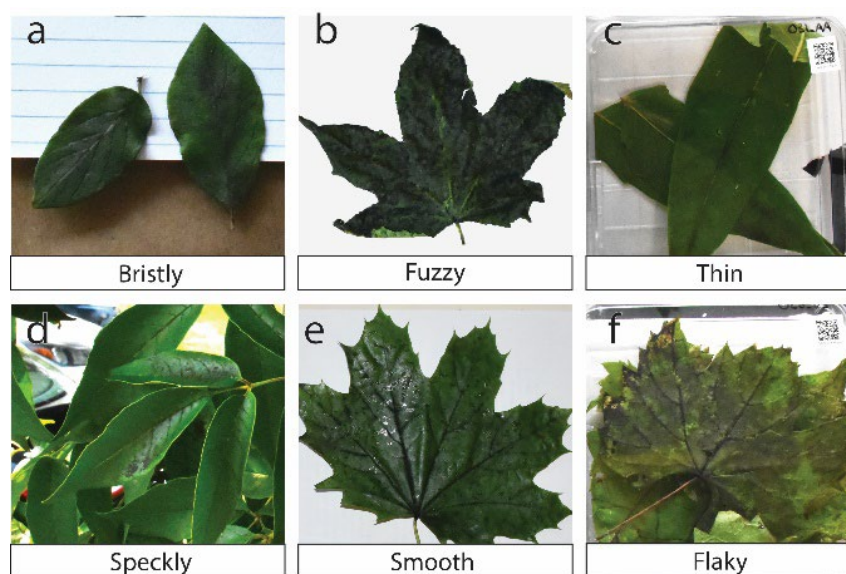


Figure 4-3. Example photos of sooty mold textures. **a.** Bristly sooty mold on *Leonicea morrowii* **b.** Fuzzy sooty mold on *Liquidambar styraciflua* **c.** Thin sooty mold on *Ailanthus altissima* **d.** Speckly sooty mold on *Fraxinus americanus* **e.** Smooth sooty mold on *Acer platinoides* **f.** Flaky sooty mold on *Vitis riparia*

Light microscopy

Early sooty mold colonies in relation to *L. delicatula* is often concentrated at leaf veins. Examination by dissection scope revealed that the distribution of sooty mold mycelia was concentrated at leaf veins both before sooty mold symptoms were visible to the naked eye, and when leaf surfaces were entirely covered. Mycelial mats were easily removed from leaf surfaces, and especially so in more severe samples. Removal of mycelia revealed leaves with no clear evidence of damage from fungal infestation. Sooty mold mycelia were composed of hyphae, conidia, pycnidia, and ascomata. Observation at higher magnifications of the stereomicroscope revealed hyphae formed netlike structures across leaf surfaces and entwined with one another to form a rope-like appearance along veins (Fig. 4-4).

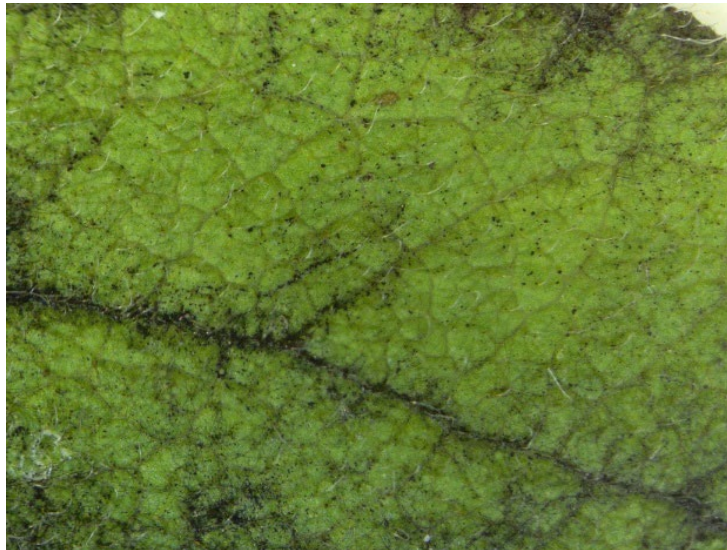


Figure 4-4. Distribution of sooty mold fungi across leaf sample of *Leonicera morrowii* at 1.2 magnification. Sample collected in Juniata, Pennsylvania on Sept 03, 2021. Sooty mold coverage on whole plant was rated (2), sooty mold severity on whole sampled lead was rated (5). Sooty mold texture was described as “smooth”. Colonies and discoloration are visibly concentrated along leaf veins.

There were four dominant spore and conidia types. The first of these was pale brown stauroconidia with 3-4 arms, each with 2-4 septa arising from a central cell. Conidial arms were 14-23 μm long, 3.5-4 μm wide at their base, and tapered to 1.2-2 μm wide at their tips (n=5) with 2-4 septa per arm. Conidial arms were slightly constricted and darker at septa (Fig. 4-5a). These stauroconidia were present in every specimen examined and were abundant in many samples.

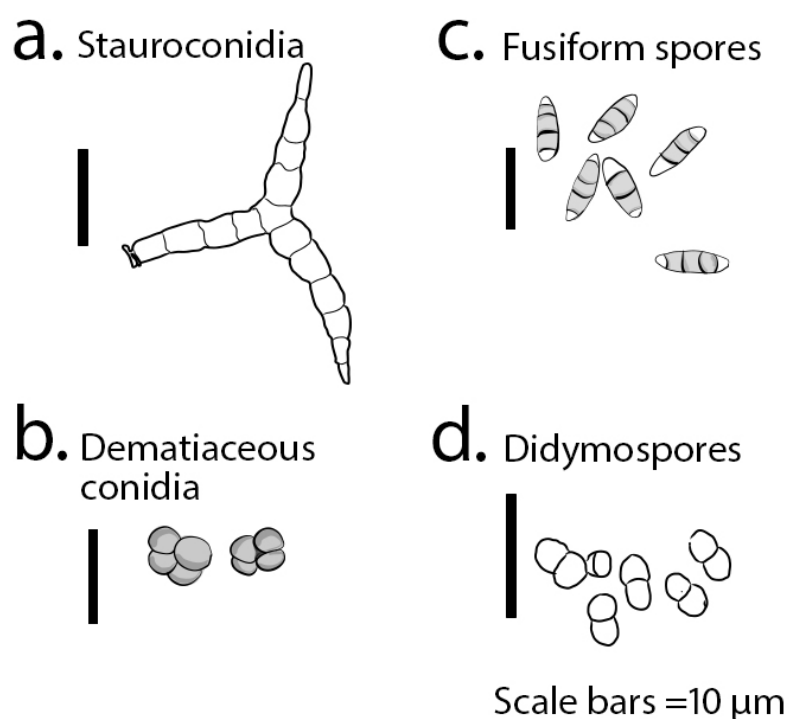


Figure 4-5. Sketches of common spores and conidia. **a.** Stauroconidium found on *Vitis riparia*. **b.** Dematiaceous conidia found on *Leonicea morrowii*. **c.** Fusiform spores found on *Acer platinoides*. **d.** Didymospores found on *Acer platinoides*.

The second most common and abundant were dematiaceous conidia which were 4-7 μm wide (n=10) and constricted at longitudinal and transverse septa. Septa most often divided these spores into four cells, which were 2.2-3.2 μm wide (n=10) (Fig. 4-5b). These conidia commonly occurred in clusters and were most abundant in specimens

on which the texture of the sooty mold was observed to have more depth, especially those described as primarily “fuzzy” or “speckly”. They were far less common and abundant in sooty mold specimens described as “bristly” or “thin” (Table 4-4, Table 4-5).

Table 4-4. Rating scale for the severity of sooty mold on a given leaf- adapted from Bovi et al. (2004) based previous observations of Spotted lanternfly associated sooty molds.

Sooty mold Severity	n	<i>Trichomerium</i> -like				Capnodiales-like		
		Stauroconidia	Upright conidiophores	Setose perithecia	Fusiform spores	One septate spores	Dematiaceous conidia	Elongate pycnidia
0	7	1.00	0.43	0.00	0.14	0.00	0.43	0.00
1	5	1.00	0.40	0.20	0.00	1.00	0.40	0.00
2	5	1.00	0.40	0.60	0.20	0.80	0.80	0.00
3	7	1.00	0.43	0.43	0.00	0.43	1.00	0.14
4	5	1.00	0.60	0.00	0.00	0.80	1.00	0.00
5	7	1.00	0.43	0.29	0.14	0.86	1.00	0.00

Table 4-5. Proportion of sooty mold samples of each texture with at least one observation of key diagnostic characteristics. Sooty mold textures listed along leftmost column, number of samples with each texture noted in column “n”. Proportions range from 0 to 1, with “n” as denominator.

Sooty mold Texture	n	<i>Trichomerium</i> -like				Capnodiales-like		
		Stauroconidia	Upright conidiophores	Setose perithecia	Fusiform spores	One septate spores	Dematiaceous conidia	Elongate pycnidia
NA	7	1.00	0.43	0.00	0.14	0.00	0.43	0.00
bristly	5	1.00	0.40	0.00	0.00	0.80	0.60	0.00
flaky	6	1.00	0.33	0.33	0.00	0.83	1.00	0.00
fuzzy	3	1.00	1.00	0.00	0.00	0.67	1.00	0.00
smooth	8	1.00	0.38	0.25	0.25	0.63	0.88	0.00
speckly	5	1.00	0.20	0.80	0.00	0.80	1.00	0.20
thin	2	1.00	1.00	0.50	0.00	1.00	0.50	0.00

A dark brown setose perithecium was removed from a leaf surface and dissected during slide preparation. Opening the perithecium exposed fusiform ascospores with 3-4 septa. These spores were 7.5-9.2 μm long, and 2.6-2.9 μm wide at their centers (n=5).

Both ends of these spores were narrowly rounded and hyaline, while their centers were a very pale brown (Fig. 4-5c). These spores occurred relatively commonly otherwise, especially in specimen collected later in the season. Finally, we found some ovate pale conidia, with slight constriction at septa, which were 4.9-5.7 μm long and 2.5-3.3 μm wide (n=5). We observed some evidence that these conidia were produced in chains (Fig. 4-5d).

Round pycnidia were observed in several specimen, though they were slightly more common in specimens with less severe sooty mold. These arose directly from hyphae, and were pale to hyaline, and globose with no distinct neck, though the rim of ostiole rose above their tips 13.5-28 μm long, 12-29 μm wide (n=5) (Fig. 4-6a). Elongate pycnidia, however, were most common in specimens with severe sooty mold with was described as “bristly”. They were gregarious, 35-92 μm long black-brown pycnidia, and appeared synnematosus. Slightly swollen at just above base (13-16 μm wide at base), tapering towards tip (5-9.5 μm wide at tip (n=5)), with a slight flare at their ostiole, which was surrounded by hyaline hyphae (Fig. 6b). We also observed conidiophores arising at right angles from hyphae, which were hyaline and tapered from base to tip (Fig. 6c).

Arising from mycelial masses, we observed dark brown perithecia, which were flask-shaped, about 45-72 μm length, 35-68 μm wide (n=10), with slight tapering at neck. (Fig. 7a-b). Setose perithecia were also common, and were dark brown and spherical, with constricted necks, 18.5-27 μm wide at the broadest point of the flask, and 20.5-27 μm long (n=5). Setae, which arose from the mycelia surrounding the perithecia and the perithecia themselves, were erect to slightly curved, and septate (Fig. 8a).

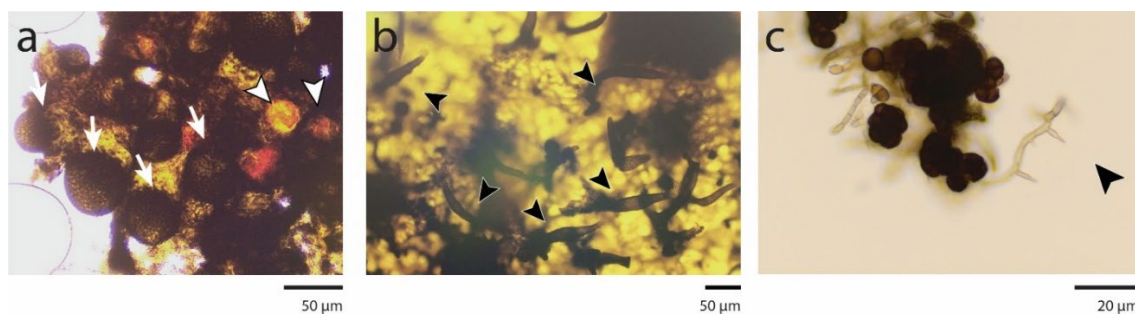


Figure 4-6. Conidial structures. **a.** Arrows indicate perithecia, arrowheads indicate round pycnidia. Removed from *Juglans nigra*. **b.** Arrowheads indicate elongate pycnidia. Removed from *Juglans nigra*. **c.** Arrowheads point to conidiophores. Removed from *Morus rubrum*.

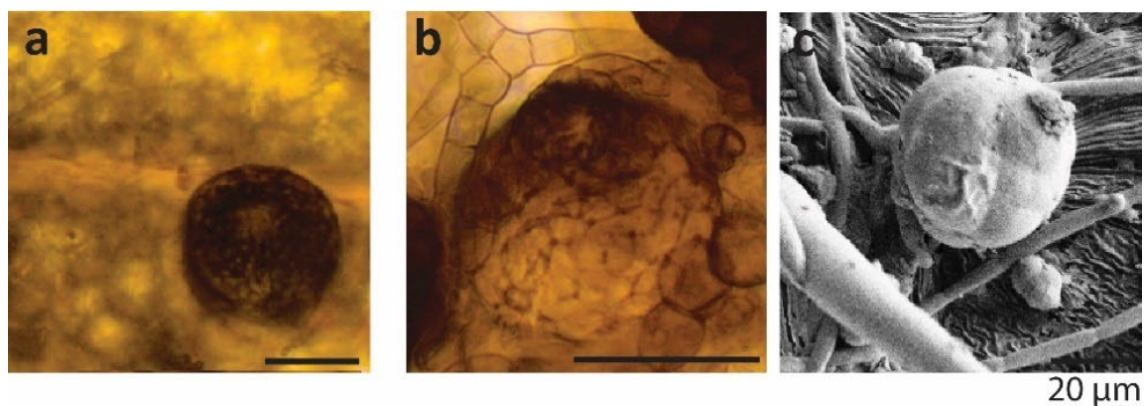


Figure 4-7. Smooth perithecia. **a.** Perithecium arising from surface of *Carya glabra*. **b.** Perithecium emerging from hyphal networks on *Liquidambar styraciflua*. **c.** Smooth perithecium arising from *Ailanthus altissima*.

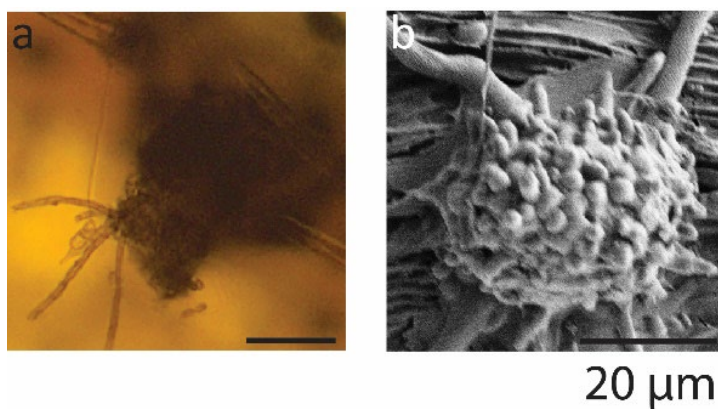


Figure 4-8. Setose perithecia. **a.** Setose perithecium arising from *Ailanthus altissima* leaf sample. **b.** Setose perithecium arising from *Ailanthus altissima* leaf sample.

Field emission scanning electron microscopy (FESEM)

13 samples of sooty mold were selected for examination with FESEM, from six plant species—*Vitis riparia*, or river grape (n=2), *Ailanthus altissima*, or Tree of Heaven (n=7), *Morus alba*, or white mulberry (n=1), *Leonicera morrowii*, or Morrow's honeysuckle (n=1), *Liquidambar styraciflua*, or American sweetgum (n=1), and *Acer platanoides*, or Norway maple (n=1)—with a mean sooty mold severity of 1.24 (0-5 scale). Fixation appeared to preserve original attachments and distribution of hyphae on the leaf surface, based on comparisons to light microscopy images. Fungal characters did not collapse and appeared well-preserved.

Even leaf surfaces with very little to no sooty mold visible to the naked eye presented with fungal characters which indicated the early development of sooty mold, consistent with our observations of specimens at low magnification by stereomicroscope. We often found hyphae wrapped around leaf trichomes, which in samples with little sooty mold created the appearance of latticework connecting trichomes across the leaf surface (Fig. 4-9a). As other studies have found, we observed no evidence of penetration into leaf (Kim 2017).

We observed stauroconidia matching the description of those found in light micrographs in great abundance arising from hyphal networks (Fig. 4-9c). Higher magnifications revealed that the hyphae these conidia arose from were round, with slight constrictions at septa, and had textured surfaces. At higher magnifications, stauroconidia were observed in development (Fig. 4-9d). We also observed a clamp connection among these hyphae (Fig. 9c). There were also clusters of dictyospores which resembled those

found in light microscopy, but they were less abundant in FESEM images (Fig. 9c).

Finally, we did observe smooth perithecia (Fig. 7c) and setose perithecia (Fig. 8b) which resembled those observed in light micrographs.

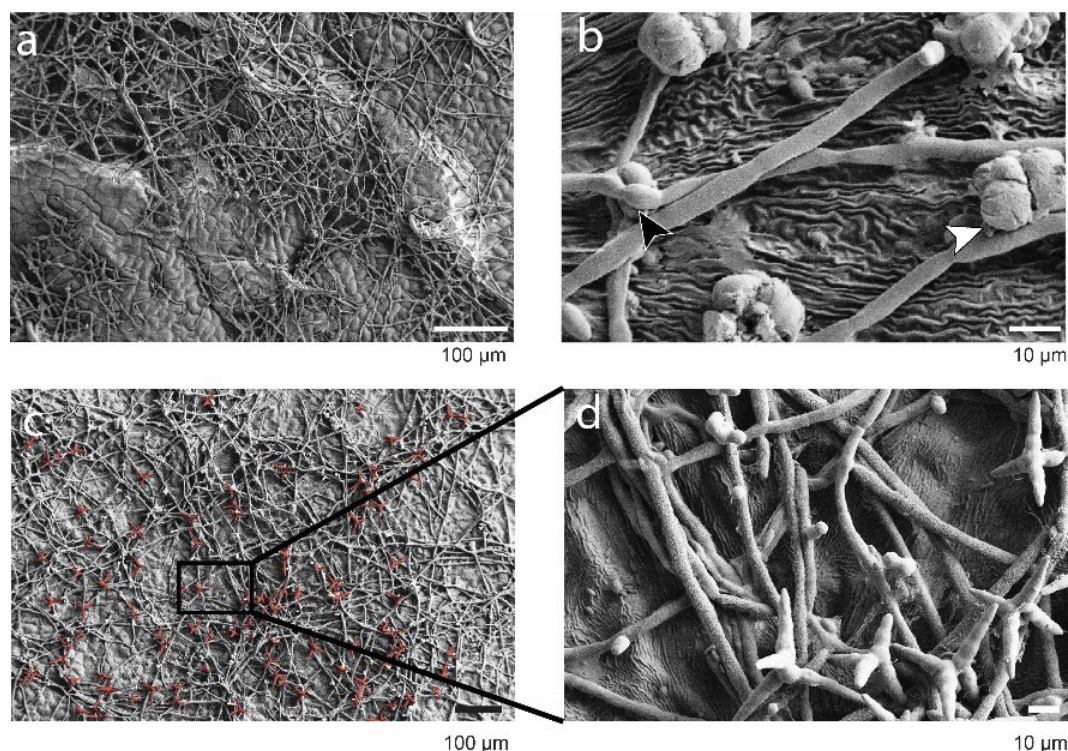


Figure 4-9. Field emission scanning microscopy of sooty mold hyphae on adaxial surface. **a.** Hyphal networks on *Leonicera morrowii*. **b.** Sooty mold fungi on *Ailanthus altissima*. Clamp connection indicated by black arrowhead. Dictyospore indicated by white arrowhead. **c.** Artificially recolored stauroconidia arising from hyphal network on *Acer platinoides*. **d.** Higher magnification of figure 9c. Stauroconidia developing from conidiophores on textured hyphae on *Acer platinoides*.

Discussion

In this study, we demonstrated morphotypes and symptomology of *L. delicatula* sooty mold by light and field emission scanning electron microscopy. Both methods showed hyphal mats forming. In less severe samples, hyphae were concentrated along veins and wrapped around trichomes, while in more severe samples, hyphae covered the

entire leaf surface. These findings mirrored our observations of sooty mold symptom development beginning primarily with staining along leaf veins.

The main characteristics of the sooty mold examined were highly abundant stauroconidia. Stauroconidia appeared similar to those found in the genus *Trichomerium*, which is consistent with the presence of this taxon in our DNA-sequence-based findings from our previous studies. In addition, upright conidiophores appeared similar to those found among *Trichomerium*, as well, as did the setose perithecia which were found in light and FESEM micrographs (Hongsanan et al., 2016). Additionally, the tapered fusiform spores which were dissected from a setose perithecium resemble those found in *Trichomerium* (Chomnunti et al. 2012). The consistency of measurements and appearances of these features indicates the presence of a single morphospecies of *Trichomerium* in *L. delicatula* sooty mold.

We also found clustered dematiaceous dictyospores, one-septate ovate conidia, and elongate pycnidia, which all resemble those found in the sooty mold taxon Capnodiaceae. However, unlike our findings with *Trichomerium*, the variation in appearance and size we observed does not suggest the presence of a single morphospecies of Capnodiaceae; rather may represent multiple species or even genera (Chomnunti et al 2011).

We did observe some other characters in small quantities, primarily those which appeared to be protected forms of spores. It is important to note that previous research has indicated that common epiphytic fungi rarely thrive on honeydew covered leaf-surfaces, which may explain the dominance of sooty-mold adapted genera. Similarly, it

may help to explain why our initial sample loss due to mycoparasitism was concentrated among samples collected earlier in the season, with lower sooty mold severity.

There was no obvious effect of location or plant species on sooty mold admixtures, though we did observe some differences by sooty mold severity and sooty mold texture. For example, while stauroconidia were present and abundant in all textures and severities, dematiaceous spores were rarer in specimens with sooty mold severities between 0 and 2, and more common in specimens with sooty mold with had a dimensional appearance to the naked eye, especially those described as “fuzzy” or “speckly”. Also, while there was no observed connection between plant host species and the presence of any one morphotype or character, dematiaceous dictyospores were somewhat less common and abundant in wild-type plants than in cultivated plants.

We found stauroconidia on every specimen collected in this study. Stauroconidia, as well as other characters appearing to belong to *Trichomerium* sp. were more dominant in samples with lower sooty mold severity, and on wildtype plant hosts than on cultivated plants. In contrast, the characters which resembled a mix of members of the family Capnodiaceae were more often present, and most dominant in late-season and more severe sooty mold samples, especially those taken from cultivated plant hosts.

In our previous research, we found that as *L. delicatula* and sooty mold develop, fungal communities become less diverse. This study can help to confirm that. We observed characters unlike those of sooty mold, such as club-shaped dictyospores which resembled those of *Alternaria*, and ornamented thick-walled spores, which resembled protected spores of *Puccinia*, both of which are genera containing common epiphytes and plant pathogens, and a wide variety of hyphal morphologies. In samples with more severe

sooty mold, we observed fewer of these characters, indicating that a phyllospheric habitat which is well-suited to sooty mold fungi, is not well suited to common epiphytes. This study also indicates that even as total fungal diversity is reduced, sooty mold admixtures become more diverse.

We found more consistency of admixtures within this pest complex than did other research teams in related sooty mold systems (Kim and Kim 2017). We believe this stems from our approach to diagnosing the “causal” taxa in any instance of sooty mold not as the *only* taxon found, but the most dominant one. Our previous studies using DNA amplicon sequencing provided evidence for a select group of sooty mold taxa, and established clear patterns in their relative abundances over time. In other words, they allowed for the identification of multiple dominant sooty mold taxa before sooty mold was readily visible to the naked eye, and without successful culturing. In addition, our work was bolstered by recent advances in taxonomic and morphological work in sooty mold families, namely, the recent establishment of Trichomeriaceae, the family containing *Trichomerium*, along with the sexual and asexual morphs of several new species of *Trichomerium* (Chomnunti 2012; Hongsanan 2016; Quan et al. 2020). However, we found it difficult to collect control samples in this study. The samples which we did believe to have no sooty mold on them either were shown to have early development of sooty mold colonies once we’d examined them by stereomicroscope or were parasitized before they could be further examined.

Sooty mold research has struggled with myriad taxonomic and ecological roadblocks over time. Even though the tools have been developed to help upend these, approaches

which reimagine the logic of microbial identification to fit those tools have not. Here, we seek the fungal taxa primarily responsible for sooty mold on leaves affected by *L. delicatula*. We find one morphospecies, *Trichomerium* sp., is consistently present and abundant, even though sooty mold admixtures become more complex over their development. Future work in sooty mold identification should attempt a similar approach. In addition, this paper adapts already established means of describing sooty molds and suggests two 0-5 scales for rating sooty mold coverage and severity, which would help to better categorize sooty mold symptomologies between sooty mold systems.

In all samples with mild sooty mold severities (0-2), nearly all hyphae, conidia, and spore-forming structures resembled those of *Trichomerium* sp., especially in samples taken from *Ailanthus altissima*. Even though a single sooty mold fungus cannot be tied to *L. delicatula*, this *Trichomerium* sp. may still be diagnostic of the presence of *L. delicatula*, even before the insects themselves, or their associated sooty mold, is easily detectable in the landscape by typical means of visual scouting. This suggests that the presence of this *Trichomerium* sp. could serve as an early detection tool for *L. delicatula* in wild-type plants, especially *Ailanthus altissima*. At present, it is most difficult to detect new populations of *L. delicatula* early in the season, before and the in liminal environments *Ailanthus altissima* most prefers. However, given the current limits of monitoring programs, these are precisely the conditions under which new monitoring tools would be most useful.

There are limitations to a detection method such as the one we have suggested, namely that morphological examinations of samples like this can be expensive, especially in terms of processing time. In addition, our findings suggest that sooty mold fungi

diversify as sooty mold becomes more severe but indicate that the original colonizing species would still be present on the leaf surface, even when visibly obscured by the mix. A probe to test for a molecular signature of this *Trichomerium* sp. could overcome these two limitations, which would support early detection when low insect population densities hinder scouting, and later work confirming recent presence of adult insects when populations move. Future work should work to isolate and describe the *Trichomerium* sp. found in association with *L. delicatula* sooty mold to develop and test molecular probes for it, and to better establish its biogeography.

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Chapter 5

Ecologically unequal exchange of biological invasion risk: a proposed conceptual framework.

The success of biological invasions are as much a sociological phenomenon as ecological, yet research addressing them as such has been rare, and oversimple. In recent years, frameworks describing the process of biological invasion have been developed and operationalized, fostering new opportunity for global political-economic analyses of invasion ecology (IE). Here, I use an integrative review of literature to construct a holistic socio-ecological conceptual framework of biological invasion. To start, I provide a brief overview of the field of invasion ecology, then summarize the findings of a review of key and recent literature in the form of a generalized model of biological invasion. I then summarize the findings of a review of key and recent literature in ecologically unequal exchange (EUE) with a discussion of EUE's mechanisms and their contexts in the world system. The conceptual framework is constructed by inferring the interactions of EUE with biological invasion. The proposed framework posits that the risk of incidence and impact of biological invasions is unequally exchanged, and that unequal exchange obscures not only local but total global incidence of biological invasion. It also suggests that greater methodological flexibility in EUE can be achieved by a synergistic application of world systems theory.

Introduction

Biological invasion¹ poses a large and growing threat to human society and the natural world. Bioinvasions undermine agricultural production, human disease management, and infrastructure (Bradshaw et al. 2016). They have also been shown to contribute to biodiversity loss, habitat loss, and even climate change (Ignace et al. 2018; Kenis et al. 2009). The rate of invasion incidence has been accelerating at least since the 19th century and is expected to continue to do so (Ricciardi 2007; Seebens et al. 2017). This raises questions about the drivers of invasion, answers to which are expected to provide critical insight to combat specific invasions, as well as the whole of the problem at the global scale.

Biological invasion is a form of environmental damage which is as influenced by society and political economy as it is by the environment; bioinvasions can therefore be understood both ecologically and socially. However, these two aspects of biological invasion are often conflated in research, political, and regulatory spheres. For example, to become invasive in an ecological sense, a propagule population must expand its *habitat*, but bioinvasions are often interpreted and described relative to political borders (Nuñez & Pauchard 2010). Bioinvasions can cause real environmental damage, but human urgency to prevent or mitigate that damage is framed in at best economic and at worst nationalist/ethnocentric terms (Simberloff 2003; Stoett 2010). The movement of

¹ In this article, ‘biological invasion’ refers to the process and phenomenon, whereas ‘bioinvasion(s)’ are individual instances of its occurrence. When discussing bioinvasions, I refer to the populations which have entered any stage of this process.

propagules is definitionally human-mediated in biological invasion, and their subsequent success/failure is determined by human intervention (Tassin & Kull 2015). But it is also influenced by both “natural” and the anthropogenic environmental conditions (Jeschke et al. 2013; Meurisse et al. 2019). Combatting the rising prevalence and damage of bioinvasions requires the development of interdisciplinary theory which disentangles the social and the ecological, and which defines the social-ecological.

Invasion ecology² seeks to explain the process of human-mediated biological invasion to develop tools that predict, monitor, and respond to bioinvasions to reduce their impacts to habitats and humans. However, the current state of invasion ecology research has not provided sufficient support to policymakers, regulatory bodies, or other stakeholders to support effective and efficient methods of prevention, management, and mitigation (Simberloff 2014). In response to this and epistemological and ontological critiques, there have been recent advances in theoretical and conceptual approaches to the biological and ecological principles governing invasion. In recent years, ecologists have developed new hypotheses and conceptual frameworks explaining trends and paradoxes in invasion ecology and exploring links to other ecological sub-disciplines such as community and disturbance ecology (Gurevitch et al. 2011; Pearson et al. 2018; Pintor et al. 2011; Shea & Chesson 2002). These frameworks vary in

² ‘Invasion ecology’ is the field of study which address biological invasion. I use ‘ecology of invasion,’ however, to describe specifically the ecological contexts of biological invasion.

intended use and audience, but a few present new opportunities for greater interdisciplinarity in invasion ecology.

As in other integrative fields, there is tension between the natural sciences, which argue socio-ecology work in invasion ecology lacks deep ecological theory, and the social sciences, which argue that natural scientists oversimplify sociological contexts (Richardson et al. 2008; Stone-Jovicich 2015; Vaz et al. 2017). A great deal of invasion ecology research from the natural sciences is interdisciplinary only in that it considers management, such as the regulations and collaborations that are required for a successful response to bioinvasions (Burgiel 2020; Larson et al. 2011; Robertson et al. 2020). Truly integrative approaches from the natural sciences rarely address global invasion ecology as a whole, instead focusing on regional landscapes or specific bioinvasions (Ferreira-Rodríguez et al. 2019; Epanchin-Niell et al. 2010). Where more global views are taken, they often simplify the process of invasion to metrics representing the transportation and/or introduction of propagules, but not the success rate or speed of establishment or spread afterwards (Early et al. 2016; Paine et al. 2016). Many use countries' volumes of trade or modelling of their ecological niches to represent risk without accounting for inequality in economic and political power (Eschen et al. 2015; García-de-Lomas & Vilà 2015). Presently, very little integrative work holistically examines biological invasion as a process within a social-ecological framework.

A global political-economic approach to the entire process of biological invasion has not been undertaken. Comprehensive approaches have been hindered by notoriously incomplete global datasets of invasive species and by a lack of theoretical and conceptual work in invasion ecology (Ricciardi et al. 2000; Colautti & MacIsaac 2004). Arguably,

integration of theories in invasion ecology with those that have emerged from environmental sociology could improve the predictive and diagnostic potential of invasive ecology by closing conceptual gaps and help overcome many of the critiques lobbied at the field (Vaz et al. 2017). Recently, conceptual work in invasion ecology has emerged in response to these challenges, offering an opportunity for renewed attempts to develop and frame interdisciplinary theory addressing the social-ecological contexts and processes in biological invasion.

In this article, I conduct an integrative literature review to construct the first social-ecological conceptual framework of biological invasion. I first conduct a review of invasion ecology and related literature to develop a theoretical model of biological invasion based on an existing model (Blackburn 2011), amended with additional literature, which establishes the mechanisms, characteristics, and contexts of biological invasion. I then conduct a review of ecologically unequal exchange theory (EUE) in the world system. EUE is an environmental sociology theory which contends that access to natural resources and environmental waste sinks flows asymmetrically from poorer to richer countries. EUE's best developed methodologies are not well-suited to integrative theoretical work, so I examine it synergistically with world systems theory from which EUE originally emerged, and which offers more mechanistic and conceptual grounding. The findings of these two reviews are then integrated. A conceptual framework emerges which suggests that the risks of incidence and impact of biological invasion is unequally exchanged. This conceptual framework offers one hypothesis to explain why orthodox invasion ecology, which does not consider social, economic and political forces, often

fails to predict trends in invasion or provide sufficient decision-support, and a path towards interdisciplinary research in invasion ecology.

Methods

Key and recent academic and grey literature regarding the ecology of biological invasions was gathered by key word search (e.g. *invas**, *introduc**; *alien*; *+species*, *organism**, *population**) from multiple databases³ (e.g. Web of Science, EBSCO, ProQuest, CAB abstracts), and key journals (e.g. *Biological Invasions*, *Conservation Biology*, *Trends in Ecology and Evolution*), along with searches in Google Scholar and Google News. This was followed by a similar search of key authors (e.g., Daniel Simberloff, Richard N. Mack, Vojtěch Jarošík, David Richardson). Finally, I searched from within references lists of uncovered articles until a point of saturation. An overview of research trends and theoretical consensus in invasion ecology is provided.

Based on findings from the literature search, I amended an existing framework to include additional mechanisms, contexts, or resolutions to paradoxes to each step in the invasion process. I selected Blackburn et al.'s Unified Invasion Framework (2011) (UIF) as the basis for my model of the ecology of invasion because it addresses many of the most common critiques of the field. The UIF offers well-defined, process-oriented

³ Because COVID-19 was emergent at the time literature was gathered, access to print resources and key library services were restricted—some literature may have been missed as a result.

terminology, which are integrated with characteristics of invasive populations (rather than individuals or species), environmental conditions, and potential human interventions, and which is organized into stepwise/non-binary flow frame (Colautti & MacIsaac 2004; Lidström et al. 2015, Warren 2007). I aimed to maintain and augment these strengths by adding literature found in the review from more recent years and parallel subdisciplines of ecology to construct my amended model of biological invasion.

I then conducted a search of literature relating to ecologically unequal exchange theory in electronic databases (e.g. ProQuest, WebofScience, HeinOnline, Annual Reviews) and in key journals (e.g. Environmental Sociology, Journal of World-Systems Research, Comparative Sociology), using key words (e.g. ‘environment*’, ‘ecol*’, ‘climate’, and/or ‘socio*’, ‘econom*’, ‘globaliz*’), followed by searches within reference lists of uncovered articles. I summarized the fundamentals of EUE and then compared them to Wallerstein’s seminal work describing the world system (1974) to enumerate the mechanisms and characteristics of EUE in the world system. Empirical examples of EUE were collected from found literature and related to these mechanisms and characteristics.

I conducted stepwise comparisons between the amended model of biological invasion and the mechanisms, characteristics, and examples of EUE in the world system. The conceptual framework is arranged in the order of the stages of biological invasion from in the amended model of biological invasion. It describes the steps and contexts of biological invasion which could be viewed through an EUE lens, and the implications of that view on the incidence and impact of bioinvasions.

Finally, I conclude by summarizing the ecological and political-economic implications of my findings. I then offer an example of a specific bioinvasion in which

every known step in its invasive history matches the predictions laid out in the conceptual framework of biological invasion risk.

Results

Invasion Ecology

Trends

Invasion ecology aims to understand why biological invasion occurs successfully in order to develop predictive, preventative, and mitigative practices. Researchers have struggled to predict the characteristics that determine the invasiveness of populations⁴. As a result, in recent years more work has focused on characteristics determining habitat invasibility⁴, with particular emphasis on large-scale modelling of ecological factors (Lonsdale 1999; Mack et al. 2000; Pearson et al. 2018; Thuiller et al. 2005). In part, this particular trend is driven by growing clarity that biological invasion is intricately linked with the some of the today's most critical global environmental crises—biodiversity loss, habitat loss, and climate change. Bioinvasions threaten biodiversity and habitats through competition, predation/parasitism, and hybridization with native species (Bellard et al.

⁴ 'Invasiveness' refers to the behavioral and physiological characteristics of a population that influence its success at colonizing new habitats and resisting counter pressures.

'Invasibility' refers to the ecological and sociological characteristics of the habitat that permit or prevent successful by a bioinvasion.

2016; Tilman et al. 2017). Habitat loss and biodiversity loss can then support the success of bioinvasions because disturbed habitats offer open niches to introduced populations, and because less rich communities are less likely to exhibit competitive resilience to colonization (Norton & Warburton 2015). As temperatures and weather patterns change, novel ranges open to biological invaders when existing biomes shift, and new biomes emerge (Jarošík et al. 2015). In turn, in some instances, bioinvasions can disrupt carbon cycles by stressing or killing flora, or by introducing new forms of waste to the local ecology, further exacerbating climate change (Ignace et al. 2018). These connections have encouraged attention to modelling the distribution and effects of landscape characteristics in regards specifically to biological invasion, and generally to environmental damage.

Another body of inquiry regarding invasion is driven by the intent to explain one of two ecological paradoxes of bioinvasions. The first paradox is that invasive organisms are not adapted to the local conditions, yet out-compete locally adapted populations. The biotic resistance hypothesis offers that even if organisms are not adapted to a novel range, they can be successful in habitats with less diversity, assuming that the diversity of their native competitors will also be lesser (Barney & Whitlow 2008). Another hypothesis to explain this paradox, the Anthropogenically-Induced Adaptation to Invasion (AIAI) hypothesis, posits that because human activity shapes the local habitat so sharply, an organism adapted to any human-adjacent landscape is better equipped to invade any other human-adjacent landscape (Hufbauer et al. 2012). The second paradox is that founding

populations⁵ are restricted in number yet adapt and thrive despite their reduced gene pools (Keller et al. 2011). Recent work agrees that genetic bottlenecks limit bioinvasion success but suggests that it can be overcome by populations with sufficiently large propagule pressure (Cassey et al. 2018). It has also been suggested that many habitats harbour an 'invasional debt' in which founding populations limited by genetic bottlenecks lag between introduction and establishment until subsequent introductions create sufficient propagule pressure (Simberloff 2014). Successive introductions are not just possible, but likely, because the trade connectivity is enormous and asymmetric, meaning that for any habitat, transportation of previously introduced bioinvasive species is more likely than the transportation of any other species (Garnas et al. 2016). These explanations indicate that to correctly interpret bioinvasion, the 'human' cannot be separated from the 'natural'.

Globalization, in one form or another, has been the point of focus for researchers addressing broader trends of biological invasions. It's generally accepted that rates of invasion, driven by transportation of propagules, are influenced by the volume and speed of trade (Meyerson & Mooney 2007). It's also been shown that trade correlates with biodiversity loss, which may promote the success of bioinvasions (Lenzen et al. 2000).

⁵ The founding population is the first generation of a bioinvasion transported to a new range. It can also be referred to as the 'propagule'. 'Propagule pressure' refers to the relative size of the founding population, and includes all individuals that were introduced, even if introduction occurred over several events.

However, conditions of globalization other than trade have rarely been addressed in relation to invasion ecology.

Critiques

A review of invasion ecology literature found as many critiques and debates as points of consensus in theory and frameworks. Many critiques stemmed from how invasion ecology has struggled to clearly define its foci and terminology. Research foci regarding both the incidence and impact of biological invasions is critiqued as being too anthropocentric, too economic, and too aligned with the hegemony (Tassin & Kull 2015; Vaz et al. 2017). These biases potentially cause swathes of biological invasions to be disregarded in the literature, such as those affecting non-commercial species (Oliva et al. 2016), micro-organisms (Mallon et al. 2015), and those primarily impacting developing countries (Nuñez & Pauchard 2010). It is suggested that these biases are considered when making broad estimations regarding biological invasion.

Another common critique is that invasion ecology often uses binary terms, or operationalizes terms in binary ways, to describe bioinvasions ('invasive' / 'non-invasive'; 'transported' / 'intercepted'). Some of the concern about these false dichotomies is purely ontological—binary terms are poorly defined and cannot be consistently understood across or even within fields, nor can they be convincingly operationalized (Colautti & MacIsaac 2004). It's also argued oversimplifying the invasion process can exacerbate issues of anthropocentrism (Stoett 2010), obscure potential mitigative interventions (Warren 2007), remove potential actors from the conversation

(Lindstrom et al. 2015). Best practices include the use of process-oriented, well-defined terms with non-binary states where possible. Blackburn et al.'s (2011) framework, on which this analysis is based, met the majority of challenges for best practices.

The Amended Framework of Invasion

Blackburn et al.'s original unified framework of invasion (2011) uses process-oriented terms to define biological invasion as a non-linear process and situates it in context with the environmental and biological conditions that influence its texture, opportunities for human action/inaction, and population characteristics of bioinvasions throughout the process. The contexts included in the framework are intended to be informative for the development and assessment of proactive and reactive planning. For the purposes of this article, the framework's primary strength is its comprehensive and flexible scaffolding. The framework describes stages of biological invasion, with more specific steps within each. The steps offer many paths through the process of biological invasion, giving each potential propagule to fail at any stage, or to succeed to various degrees. It is this scaffolding that permits its amendment.

In my amended model of biological invasion, I included additional literature that might clarify steps or help explain the contexts influencing them (Fig. 5-1; Table 5-1). Transportation of propagules ('A': Fig. 5-1; Table 5-1) occurs both accidentally (e.g., propagules "hitchhike" on material goods and packaging) and intentionally (e.g., propagules are brought to the novel range for aesthetic or commercial purposes) (Hulme et al. 2008). The sanitation quality of goods and packaging by exporters is determined by

Table 5-1. Amended unified framework of invasion. Derived from Blackburn et al. (2011)

Stage or (Step)	Mechanisms	Citation	Contexts and Complications	Citation
Transportation	a propagule population is moved beyond its native geographic range by human means.			
(A) Propagules are moved and survive transportation.	Imported commodity intentionally moved.	Hulme et al. 2008	When risk is uncertain, under-regulation is more common than precautionary principle.	Foster 2009
	Transported by vector or as stowaway/contaminant.	Hulme et al. 2008	Responsibility & cost of sanitation shifted onto exporters.	Waage & Mumford 2008
Introduction	the movement of a transported organism or population into the landscape of a novel range with suitable a/biotic conditions for survival.			
(B0) Introduction is prevented after transportation.	Interdiction by importers.		Prevention requires investments even given uncertainty.	Simberloff 2014
	Unsuitability of abiotic/biotic conditions in the novel range.		Anthropogenically-induced adaptation to invasion	Hufbauer et al. 2012
(B1) Organisms are introduced but prohibited from dispersal.	Containment measures such as quarantines.		Quarantines must be well-sealed and pro-active.	Larson et al. 2011
(B2) Organisms are somewhat restricted from dispersal	Limited containment conditions, such as cultivation.		Populations can later escape or adapt.	Sims & Finoff 2013
(B3) Individuals/populations are immediately released into the wild.	No containment.		Poor detection or estimation of threat.	García-de-Lomas & Vilà 2015
Establishment	Introduced individuals or populations survive in the landscape.			
(C0) Establishment is prevented; failure to survive/thrive.	Biotic resistance hypothesis.	Barney & Whitlow 2008	Other forms of environmental damage influence invasion success.	Lonsdale 1999
	Founder effects/propagule pressure.	Cassey et al. 2018	Establishment can lag, until successive introductions can instigate it.	Simberloff, 2014
(C1) Individuals or populations establish, but do not reproduce.	Environment/population supports survival, not reproduction.	*Hallet, 2006	Bioclimatic niches can promote or prevent reproduction.	Thuiller et al. 2005
(C2) Reproduction occurs below the rate of replacement.	Environment / population doesn't sufficiently support reproduction.		Sometimes just a lag. Opportunity for action often missed.	Sims & Finoff 2013
(C3) Reproduction occurs at or above the rate of replacement.	Environment / population supports reproduction with an advantage.	*Hallet 2006	Disturbance hypothesis.	Pearson et al. 2018
Spread	Established individuals or population spread beyond initial boundaries within the introduced range			
(D0) Spread is prevented after establishment.	Well-managed of invasive populations prevent further spread.	Eschen et al. 2015	Requires incentive to control invasives—collectivist buy-in.	Epanchin-Niell 2010
	Management of quarantines.		Requires cooperation and investment from all parties.	Paini et al. 2016
(D1) Spread occurs by gradual boundary expansion.	Populations are not well managed and expand in size and geographic cover.		Fluctuating resource availability, supports quick population growth.	Davis et al. 2000
(D2) Spread occurs by “hops”, with break-off populations.	Quarantines and trade regulations are absent or unsuccessful.		Undetected or risk not taken seriously.	Garnas et al. 2016

their political and economic capacity to sanitize exports and their aversion to the risks of failed sanitation. Risks can be calculated in ecological, economic, and/or political terms, such as harm to endangered species, or to trade relationships (Holmes et al. 2017). Of organisms that are transported, abiotic conditions in the novel habitat will prevent the vast majority from surviving. Others will be successfully introduced.

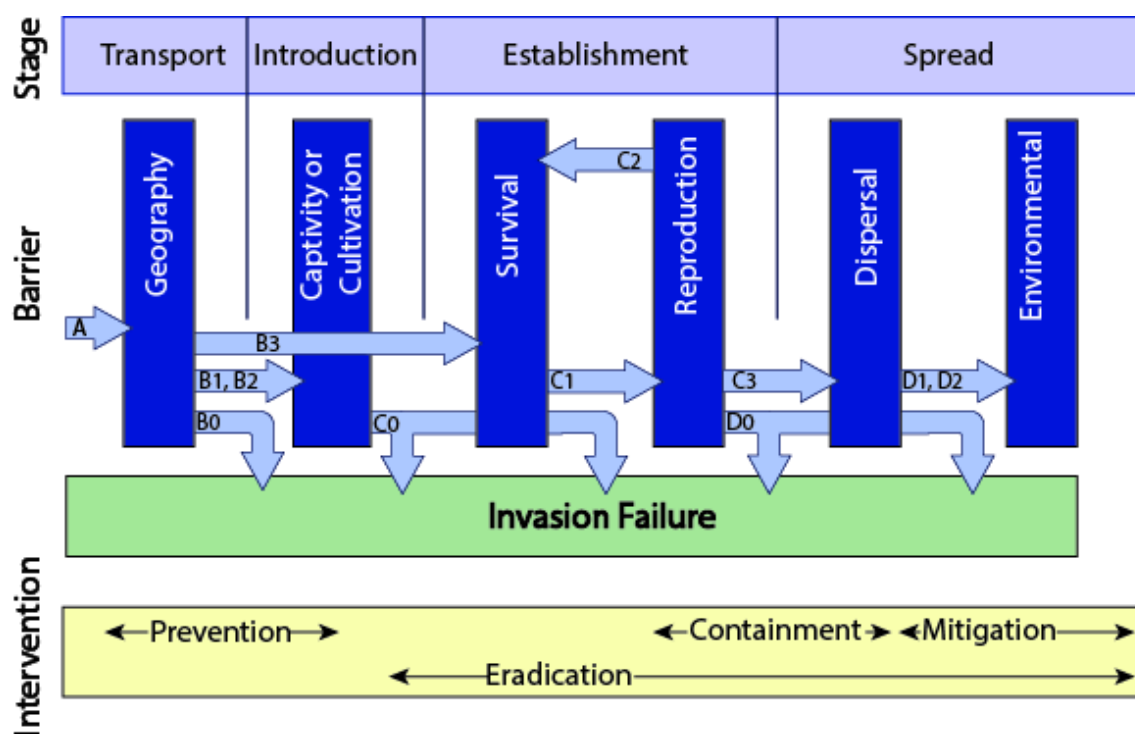


Figure 5-1. *Amended model of biological invasion.* A- Propagule survive transportation. B0-Introduction is prevented. B1- Introduced but prohibited from dispersal. B2- Introduced but dispersal restricted. B3- Immediately introduced into the wild. C0- Establishment is prevented. C1- Establishment, but no reproduction. C2- Reproduction below the rate of replacement. C3- Reproduction at or above the rate of replacement. D0- Spread is prevented. D1- Gradual boundary expansion. D2- Break-off populations.

Interdiction ('B0': Fig. 5-1; Table 5-1), or sanitation and trade bans organized by importers, is the most effective measure a receiving nation can take, but it requires

proactivity even in uncertain conditions (Simberloff 2014). Like interdiction, quarantines and containment ('B1' & 'B2': Fig. 5-1; Table 5-1) must be proactive and thorough to be effective, because failures are unlikely to be noticed until far too late, and because populations once well-contained can escape or better adapt to the novel range (Larson et al. 2011; Sims & Finoff 2013). Often, founders are not contained ('B3': Fig. 5-1; Table 5-1) because insufficiently funded/equipped programs fail to detect them, or because their potential invasiveness is underestimated by decision-makers. Blacklists, watchlists for highly invasive species, may contribute to the underestimation of invasiveness because many people incorrectly assume the lists are comprehensive (García-de-Lomas & Vilà 2015). Blacklists, of course, do not include organisms which have never before been invasive, or bioinvasions which have gone un- or under-studied.

The impacts of bioinvasions become noticeable and relevant when bioinvasions establish. The biotic resistance hypothesis argues that establishment can be limited or prevented ('C0': Fig. 5-1; Table 5-1) by taxonomic and functional diversity in the novel range, and that habitats which are disturbed by land development, climate change, or prior bioinvasions may be more susceptible (Barney & Whitlow 2008, Lonsdale 1999). Establishment can also be prevented by genetic bottlenecking amongst founders, therefore, larger propagule pressures support greater gene pools and adaptability which can be critical to success (Cassey et al 2018). Apparent failures to establish may only be lags between introduction and establishment ('C1' & 'C2': Fig. 5-1; Table 5-1) (Simberloff 2014). Lags offer opportunities for successful establishment propagule pressure grows with subsequent introductions. Because trade pathways often follow well-established relationships, re-introduction of any bioinvasion is more likely than

introductions of new species (Garnas et al. 2016). Established organisms exhibit a range of reproduction success relative to their replacement rates. They may lack some a/biotic requirements for any reproduction to occur in the novel range ('C1': Fig. 5-1; Table 5-1); they may reproduce below the rate of replacement ('C2': Fig. 5-1; Table 5-1), or above it ('C3': Fig. 5-1; Table 5-1). Comprehensive management and the conservation of diverse habitats can limit reproduction success of bioinvasions by decreasing reproduction or raising the replacement rate.

Spread can be contained ('D0': Fig. 5-1; Table 5-1) by population management of bioinvasions, which slows gradual range expansion ('D1': Fig. 5-1; Table 5-1), and/or quarantines that prevent 'hops', in which break-off populations are introduced to nearby non-contiguous ranges ('D2': Fig. 5-1; Table 5-1). Spread is influenced by many of the same sociological factors as the first wave of invasion, such as the capacity motivation to establish and execute quarantine and management plans. However, neighbouring and regional borders have greater significance in spread, so social and ecological conditions which are regional may have greater effects in spread.

Ecologically unequal exchange in the world system

World Systems Theory (WST) (Wallerstein 1974) examines the world-economy as a global division of labour among nations. This division creates countries in three distinct blocks—the core, the semi-periphery, and the periphery—whose Marxist analogues are the bourgeoisie, the landowners, and the proletariat, respectively (Fig. 5-3). Positioning in the world system is created and maintained by the diplomatic, economic, and military

power of the core and to an extent, the semi-periphery (Chase-Dunn & Grimes 1995; Wallerstein 1974).

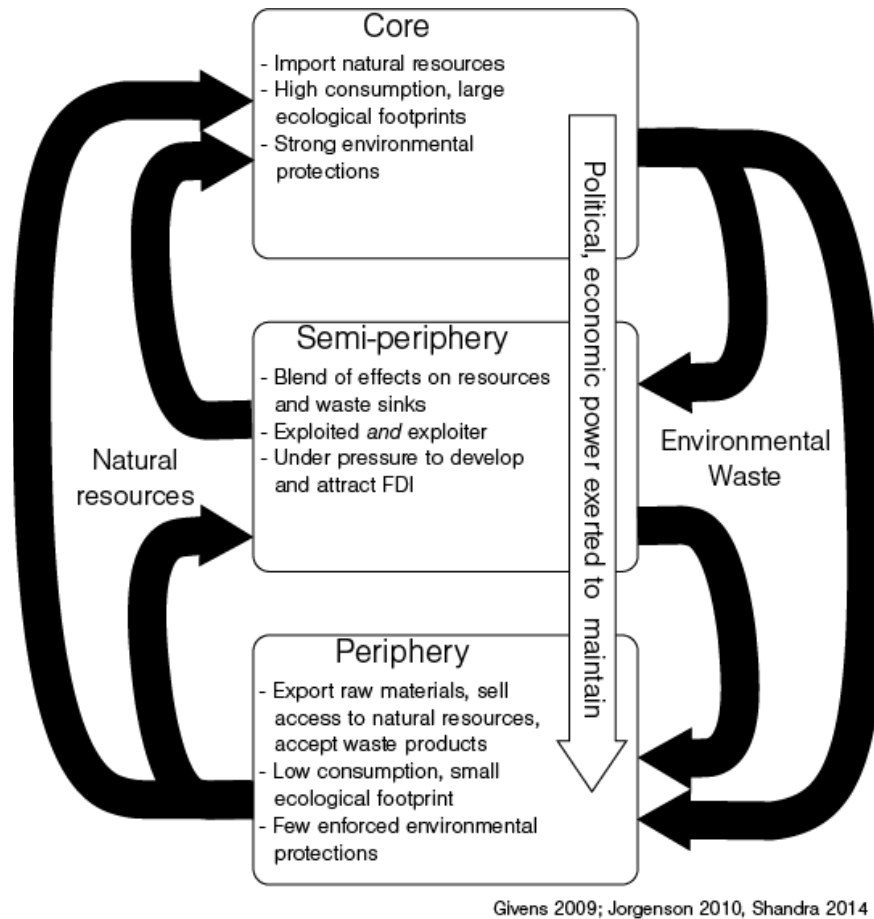


Figure 5-2. Ecologically unequal exchange in the world system: characteristics and mechanisms.

The concept of ‘unequal exchange’ is central to WST and describes the asymmetrical flow and accumulation of resources and labour across the tri-partite world system. ‘Ecologically unequal exchange’ is a more precise political-ecological examination of unequal exchange (Hornborg, 1998). Ecologically Unequal Exchange (EUE) addresses the flow and accumulation of access to natural resources and waste sinks, especially relative to consumption (Givens et al. 2019; Muradian et al. 2002; Rice

2007). EUE finds that the values (free trade, capitalization, and profit) and institutions (global governance, state machineries, and corporations) of the world market offer undue access to natural resources and waste-sinks to the core, that consumption in the core drives ecologically damaging production, and that this exploitative dynamic incurs and obscures shared costs to global ecology (Fig. 5-3) (Moran et al. 2013).

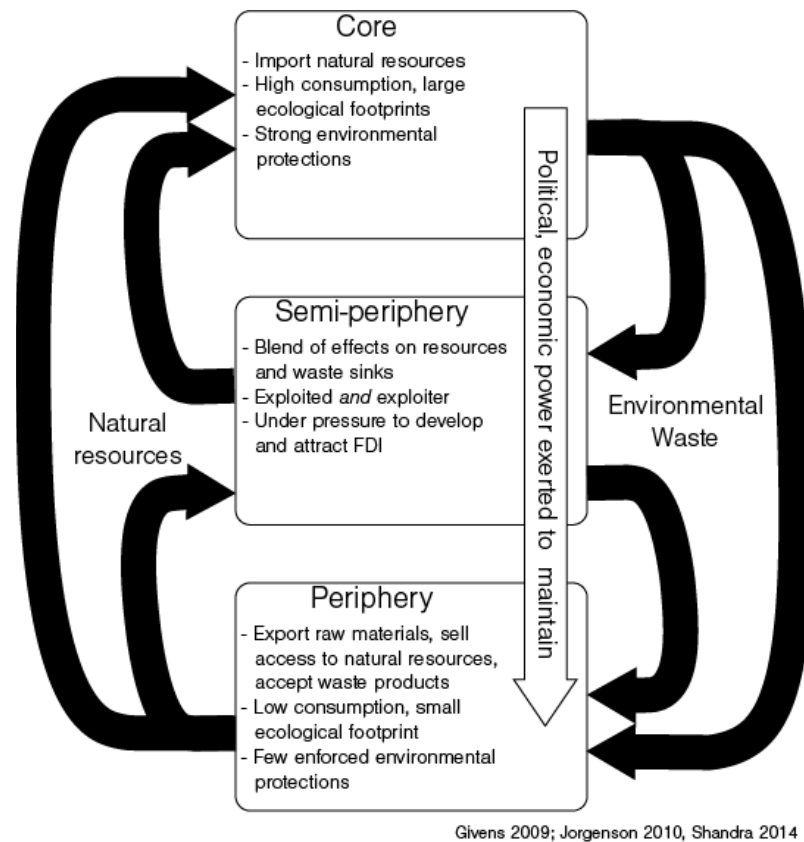


Figure 5-3. World systems theory: characteristics and mechanisms.

For example, the World Trade Organization's (WTO) 1995 Agreement on Sanitary and Phytosanitary Measures (SPS), a multilateral trade agreement which sets standards for protective measures, favours the core in several regards. For one, though the core establishes strong policies, some of which may be poorly disguised protectionism, the periphery cannot afford to bring suit in the WTO, both because of the cost of the suit

itself, and the risk suits pose to trade partnerships (Table 5-2). The periphery in turn cannot defend its own protection policies for the same reasons, and because SPS prioritizes scientific knowledge and certainty, disregarding biases in ways of knowing or funding for scientific research (Table 5-2).

Through global governance the core also can curb the power of the state in the periphery, including their ability to enact or enforce policies such as environmental protections which challenge the core or its interests (Wallerstein, 1974, Chase-Dunn & Grimes, 1995). For example, though many developing countries are reluctant to adopt trade liberalization, but they are diplomatically pressured to do so (Chang 2003). Eventually, these policies can be imposed in times of crisis by enforced structural reforms as conditions of emergency loans (Beeson & Islam 2005; Rickard & Caraway 2019). Beyond hollowing regulatory investments, they often directly limit environmental protections (McNeely & Norgaard, 1992; Salzman, Ruhl & Nash, 2015).

These dynamics between the core and the (semi-) periphery determine the degree and direction of trade specialization in the (semi-)periphery and further facilitate the accumulation of natural resources and waste sink access in the core, by limiting flexibility and agency of (semi-) peripheral economies (Muradian et al. 2002). As the core constructs the economies of the (semi-) periphery, it reinforces the inequality of economic and political power in the world system. Participation in the world-economy correlates with maintained growth rates in the core, limited upward mobility in the semi-periphery, and flat or negative growth in the periphery (Mahutga & Smith 2010). Economic power is then tightly associated with political power both within and among borders.

Table 5-2. EUE within the world system.

Mechanisms & characteristics of the world system	EUE (and related) examples	Cite
Trade liberalization policies imposed by global governance and other agreements serve the wealth of the core and undermine the protection of the periphery. ¹	SPS is biased towards the environments & economics of the core.	Nuñez & Pauchard 2010
	SPS prioritizes hegemonic ways of knowing; most scientific research and funding is located in the core.	Munyua & Stilwell 2013; Newell et al. 2019
Pressure from the core reinforces power dynamics within the world system, determining the ability to enact or enforce policies which challenge the core, its values, or its allies. ^{2,3}	Core countries enforce the most restrictive environmental protections.	Jouanjean et al. 2016; Kim 2016
	The SPS prohibits on policies which cause “undue burdens” to trade.	Das 2008
	The periphery is unlikely to challenge protections in the core or defend their own in WTO’s court.	Bown 2005; Kim 2016
Power dynamics enable the core to determine and restrict trade complexity in the periphery, creating advantageous precarity. ^{4,5}	Periphery is most reliant on environmental resources & services, and least able to afford to absorb/mitigate environmental damage.	Gonzalez 2001; Isbell et al. 2017
The actual and perceived strength of the state machinery in the periphery is diminished by core-aligned global governance institutions. ^{2,6}	States retreat from national and collaborative environmental governance and investment when enforced austerity measures are in place.	Kirsop-Taylor et al. 2020; Salzman et al. 2015
	A history of enforced austerity measures reduces fear of regulations, encourages illegal natural resource extraction and dumping.	Onyango et al. 2019
The interests and behavior of the core are aligned with those of international corporations, in contrast to those of the periphery. ²	Private labelling shapes production practices to the concerns of core consumers, rather than concerns of peripheral producers.	Bernstein & Hannah 2008; Du 2018
Economic behavior of the core drives the export of natural resources and deposition of waste. ²	Foreign land investments in periphery and create conditions in which conservation areas were difficult to establish or maintain.	Shandra et al. 2010
The consumers of the core create the greatest demand for environmentally damaging production. ^{2,7}	Trade of a specific set of ecologically pernicious luxury goods consumed primarily by the core is a driver of global biodiversity loss.	Lenzen et al. 2012
	Primary sector exports in the periphery, & proportion of those exports traded to the core associated with habitat destruction.	Jorgenson 2010
The world system is self-sustaining and self-reinforcing. The long-term effect of social, political, and economic power dynamics is greater than the sum of its parts. ¹	Environmental protection strength is correlated with positioning in the world system, even controlling for spending capacity.	Sommer & Hargrove 2020
¹ Mahutga & Smith 2010, ² Wallerstein 1974, ³ Chase-Dunn & Grimes 1995, ⁴ Chang 2003, ⁵ Muradian et al. 2002, ⁶ Rickard & Caraway 2018, ⁷ Du 2018.		

EUE focuses on the asymmetric removal of natural resources, localization of damaging production and disposal of waste products, and the relation of that asymmetry to political and economic power dynamics. Literature in this field seeks to answer whether ecologically unequal exchange is occurring, and if so, what the ecological and human impacts are, and how they are distributed. The underlying concern in this literature is that the core, whose populations drive unsustainable consumption globally, uses the semi-periphery and periphery to support that consumption by protecting the environmental benefits in the core and externalizing ecological harm outside the core. Displacement obscures the costs of consumption and permits wealthy consumers to take minimal responsibility for shared environmental damage.

Conceptual framework

Transportation

Propagule pressure per trade volume. International trade is a primary source of propagules in biological invasion. Peripheral countries engage in much smaller volumes of trade, especially imports, so it is often assumed that they are exposed to a correspondingly smaller propagule pressure (Early et al. 2016). However, EUE suggests that harm be viewed relative to consumption. As established above, the (semi-)periphery has less power to enact protective measures than does the core, and less power to enforce them, especially against the core. For example, the political economy of ‘knowledge’ and un/certainty are interpreted by the WTO in ways that advantage core-led ‘scientific

knowledge' (Johnson 2019; Newell et al. 2019). In addition, the simpler economies of (semi-)peripheral countries make them more reliant on their few trading partners than core countries are on any one of their many. Aversion to the risk of transported pests resulting in trade bans is therefore strongest in the (semi-)periphery. Therefore, *per volume of trade*, the periphery is most at risk of higher propagule pressure.

The burden of prevention. The impacts of bioinvasion at this stage are often considered in terms of sanitation costs for exporters and opportunity costs of preventative measures. EUE would predict that costs like these would be shifted from the core to the (semi-)periphery. In addition to a greater ability to protect itself from propagule pressure per volume of trade, the core can use the same dynamics to demand sanitized imports, or to exclude a non-compliant nation from the exporter market (Jouanjean, Maur, & Shepherd 2016). This suggests that the costs of the sanitation and trade bans are externalized outside the core by nature of risk aversion in the (semi-)periphery, even though in relative terms, the burden of prevention is much greater in the (semi-)periphery, which must spend a greater proportion of its economic and political capital to enforce sanitation measures (Waage & Mumford 2008).

Introduction

Actual state capacity. Barriers to introduction must be publicly managed, proactively funded, and well-equipped (Holmes et al. 2017). WST, however, notes that intranational capacity of the state is shaped by positioning in the world system. The core is advantaged

in its actual public investment in research and education. Tools developed from scientific research are therefore likely to be better adapted to the realities of the core (Pysek et al. 2008). For example, bioinvasions have been most studied in the core, so blacklists developed from the body of invasion ecology research are less effective when applied in the (semi-)periphery. Additionally, the periphery has little actual capacity to purchase and maintain physical infrastructure or human capital to monitor for transported organisms and to prevent their introduction. Detection programs in the periphery are therefore likely to be weakest (Early et al. 2016). Introduction is more successful in the (semi-)periphery because their state machineries are limited by actual capacity to create and implement protections against introduction, and in turn, least successful in the core.

Perceived state legitimacy. Effective quarantine and import sanitation requires strong public/private cooperation, and trust in the state. However, EUE finds that the conditions of positioning in the world system determine the perceived legitimacy of the state, and that those perception affect private actors' adherence to environmental protections. Enforcement of quarantines in the in the periphery contends with greater non-compliance because forced structural adjustments will have affected a larger proportion of countries in the (semi-)periphery, and non-compliance with biological invasion protections will mirror non-compliance with other environmental protections (Kirsop-Taylor, Russel, & Winter 2020). Additionally, the valuation of 'free trade' above nearly all else gives rise to the false idea that the (semi-)periphery cannot afford environmental protections, further diminishing faith in the value of quarantines (Gonzalez 2001). In contrast, the core is better aligned with capital accumulators (i.e. corporations, traders), so it will be more

able to enforce regulations across these stronger relationships (Sommer & Hargrove 2020). This suggests that external and internal perceptions of the state machinery will undermine successful enforcement in the (semi-)periphery and support it in the core.

Establishment

Trade asymmetries of propagule pressure. Establishment of a bioinvasion can occur after a long lag, given sufficient propagule pressure accumulates by way of re-introduction, which in most cases, is more likely than the introduction of a new species. WST, however, establishes that the (semi-)periphery has simpler economies and simpler trade relationships. This suggest that the effect of trade patterns on the likelihood re-introduction in support of propagule pressure will be strongest in the periphery, and weakest in the core.

Ecologically unequal exchange of disturbances. Ecological conditions such as local biodiversity and habitat disruption have strong impacts on the success of establishment. However, EUE finds that biodiversity loss and habitat disruption occur fastest in the semi-periphery, because the pressure to develop is equally applied to the periphery and semi-periphery, but the capacity to do so is greatest in the semi-periphery (Sommer & Hargrove 2020; Shandra et al. 2009). This suggests that where ecological damage is occurring the fastest, the semi-periphery, establishment of biological invasions will be most successful, and where is it least impactful, the core, establishment will be least likely.

Relative impacts. The impacts of invasions begin to accrue during establishment. EUE and WST suggest that impacts are best measured in relative terms. In the periphery individuals, communities, and state machineries are most reliant on natural resources and least capable of absorbing costs and mitigating damage (Paini et al. 2016). The periphery, and then the semi-periphery, therefore experience the greatest relative impacts of bioinvasions.

Spread

Crux of the invasion cycle. Invaded regions become sources of successive bioinvasions elsewhere. Good quarantine management can reduce the escape of break-away populations, and good population management can reduce gradual population expansion. Yet the management of these programs is governed by the same sociological conditions which affect the first three stages of biological invasion. This implies that for all the reasons the (semi-)periphery is disadvantaged in managing and mitigating the prior stages of biological invasion, it will be disadvantaged in its ability to prevent the spread of bioinvasions beyond its borders.

Spread and economies are regional. Spread most easily occurs within biomes, across neighboring and nearby political borders. Contemporary borders do not reflect the histories that created underdevelopment in the (semi-)periphery, and peripherality is distributed in larger swathes of the globe than each countries' political borders, so peripherality is somewhat regional (Frank 1969). The conditions that support invasion in

any peripheral country are more likely to also be true of neighbouring nations. This suggests that spread is occurs more easily in regions with more peripheral countries.

Spread is relational. Preventing spread requires similar measures to those which prevent transportation and strong cooperation between source countries and countries at risk and importing bioinvasions (Epanchin-Niell 2010; Paini et al. 2016). This implies that given asymmetrical positioning, the same dynamics which govern capacity and incentive in transportation will affect capacity and incentive in spread. However, WST argues that the world system affects within-block relationships as much as it does between-block relationships. Because spread and positioning are regional, this is of greater importance in spread than in transportation. Along core-core borders, there is strong incentive for the invaded nation to control the invasive population and prevent spread, and strong capacity for the uninvaded country to prevent transportation and introduction. Additionally, because economic interests of core countries are largely aligned, cooperation is unencumbered by competition. When a border is between two semi-peripheral countries, their potential for cooperation will be diminished by the competitive relationship the semi-periphery is thrust into by the core (Wallerstein, 1976). Therefore, the incentive of the source country and capacity of the at-risk country are reduced in semi-peripheral relationships. In periphery-periphery relationships, there is little economic incentive for the source country to prevent spread, because it is less likely that two peripheral nations are reliant on one another's trade. Both source and at-risk countries in the periphery also have less capacity to prevent spread, for all the reasons earlier in the model. This all

suggests that in symmetrical and asymmetrical relationships, the core is most advantaged, and the periphery least advantaged in preventing the spread of bioinvasions.

Discussion

This conceptual model seeks use an integrative review of the literature to interpret biological invasion as a waste product of trade which could be affected by ecologically unequal exchange in the world system. I find that EUE in the world system contributes to unequal risks of the import, export, and impacts of bioinvasion. Inequality in political power creates inequality in import risk per volume of trade and inequality in labor and financial burden of prevention. Global governance then limits the establishment of environmental protection policies, especially in semi-peripheral and peripheral nations, and structurally restrict those same nations' agency to challenge protections enacted in the core. The political and economic behaviour of businesses and institutions then diminishes the agility and efficacy of the protective measures that the semi-periphery and periphery *can* enact. Though the disadvantages of the semi-periphery and periphery may vary across factors, the advantageous position of the core in biological invasion does not falter at any point in the process. This is especially true when risks and their costs are understood relative to the consumption and wealth of the core.

The mechanisms and contexts of EUE affect the process of biological invasion. I also find that other examples of environmental damage governed by EUE affect the invasibility of habitats in the world system. Biological invasion is closely associated with other forms of ecological damage, including climate change, habitat destruction and

biodiversity loss, which are known to be unequally exchanged. Countries which rely on exports of natural resources are at greater relative risk of the impacts of bioinvasions, and at the same time experience higher prevalence and severity of environmental damage which supports biological invasion.

This review adds political-economic nuance to understandings of biological invasion. Where many studies have focused on single societal factors, like trade, this approach uses a body of environmental sociology to assess the whole effects of political, economic, and social power in the global spread and impacts of invasive organisms. This is, however, a first step in developing interdisciplinary theory in invasion ecology. Findings would be greatly strengthened by critique, and by contrasting applications of other social-ecological theories to invasion ecology. Global data regarding the process of recorded invasions is lacking in coverage and depth, limiting empirical testing. However, future work should attempt to empirically test the hypotheses presented in this review, whenever there is sufficient data to do so.

Interestingly, the literature review also uncovered an anecdotal example in which the posited mechanisms of unequal exchange in biological invasion shaped the transportation, introduction, establishment, and spread of the larger grain borer (*Prostephanus truncates*) in Tanzania and the region beyond. Larger grain borer (LGB), a pest of stored products, was first transported in the 1970s to Tanzania as a hitchhiker on an aid shipment in the midst of a famine. Introduction was aided by the perceived illegitimacy of state regulations—donors, with the help of several core governments, pressured Tanzania into waiving quarantines, arguing that the nation couldn't afford to

risk the time it would take to be sure of a clean shipment, and that it could be reasonably assumed that donations from “developed” countries were sanitary (Tyler and Hodges 2002). Though a new type of damage was reported by farmers in 1979, state agencies dismissed this local knowledge as mistaken until its identity was confirmed by scientists in 1981. After such a delay though, the pest had established, and eradication had become unlikely. The Food and Agriculture Organization of the United Nations (FAO) coordinated management starting in early 1984, citing a desire to prevent its entry into core countries. They established monitoring, training, and management programs. Often, these programs were based on research in ‘conventional’ commodity farming, and were therefore inappropriate, too expensive, or impossible for small-scale traditional farmers. International restrictions on trade to prevent the spread of the pest were later found to be unnecessary to prevent spread out of the region to developed countries, and ineffective for spread within the region. The restrictions did, however, reduce critical trade within the region (Farrell & Schulten 2002). As larger grain borer established and spread throughout nations in eastern and western Africa, it began to affect not only maize, an introduced crop, but stored cassava, a native staple crop as well (Quellhorst et al. 2021). Ironically, LBG now exacerbates the risk of famine as it continues to threaten staple crops and even aid shipments to communities living in poverty.

In summary, this integrative review posits that the mechanisms and characteristics of ecologically unequal exchange do affect the process of biological invasion. It offers a way to operationalize contemporary frameworks of biological invasion, while keeping up with the ever-changing scientific consensus. The review also treats EUE

methodologically as existing within the world system, lending new flexibility and applications to the theory. Finally, it suggests that social-ecological approaches to biological invasion are a strong path forward to strengthen invasion ecology, and its applications to combat the global issue of bioinvasion.

New theory and frameworks have been developed in recent years to better comprise the whole of what is known about invasion ecology; however, they have been underutilized in theoretical and empirical research. This study offers a way to operationalize Blackburn et al.'s (2011) unified framework of invasion for use in studies which intend to examine the whole process of invasion. Operationalized, the unified framework can also inform best practices for data collection, and call attention to often missed factors in documenting specific biological invasions. This article also offers a method for post-hoc amendment of the unified invasion framework to add to its utility over time. Similarly, when best management practices are inhibited by structural barriers, policy and practice can be reviewed in that context to develop more effective global responses.

This work also suggests a way to interpret ecologically unequal exchange in the world system such that the strong theoretical backbone of WST, the specificity of EUE and the methodologies of both can be fully utilized in a research application. A synergistic application of WST and EUE makes EUE more flexible and more accessible. For example, a clear means of examining ecologically unequal exchange mechanistically lends the approach to ecologists interested in applying a socio-political lens to their study system.

Biological invasions are a particularly difficult question to address in environmental policy and regulation. This integrative conceptual framework suggests that there is a portion of biological invasion made invisible to global accounts, that the significance of the problem is poorly accounted, and that precaution is undervalued relative to risk. Thus far, trade policies that are designed to prevent biological invasions have been largely regarded as failures, which has motivated the search for biological and ecological predictors of invasions. However, there may be, hidden in the unexamined sociological dynamics on biological invasions, attainable socio-political solutions. If EUE is occurring in biological invasion, then biological invasion might be reduced in its incidence and impact if justice-focused changes are made to international trade agreements, both directly and indirectly relating to biological invasion. Future research should continue to explore the socio-ecological dynamics of biological invasion.

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Chapter 6

Conclusions

The ontological issues of invasion ecology would be best served by a greater effort to study and interpret individual bioinvasions as phenomena situated within complex ecological interactions, and a greater effort to add interdisciplinarity, namely socio-ecology, to the theory of invasion ecology. In this dissertation, I provided an example for an ecological approach to a bioinvasion and proposed a wholistic socio-ecological model of biological invasion in general.

I explored the fungal ecology of *Lycorma delicatula* in order to better define the ecological impacts of the insect, and to seek potential avenues for pest management. In Chapter 2, I found that as honeydew of *L. delicatula* was deposited on the leaf surfaces of *Ailanthus altissima*, fungal communities became more similar to one another, indicating that honeydew was acting to select for the fungi that would thrive in the *L. delicatula*-modified environment. In Chapters 2 and 3, though I identified several potential sooty mold fungi, one, *Trichomerium* sp., was consistently abundant across plant species. These findings were echoed in Chapter 4, in which several sooty mold morphotypes were present, and a few were abundant, but only a single morphospecies of *Trichomerium* was present in all samples.

My findings concur with previous research which asserts that sooty mold colonies are mixed (Hughes & Seifert 2012), but my results disagree with their assertion that those

mixtures are random. The consistent presence of *Trichomerium* sp., paired with the convergence of fungal communities exposed to *L. delicatula* honeydew implies that honeydew is not simply selecting for a general swath of fungi that are capable of surviving on it, but for a specific set of fungi which are best adapted to the particular composition of *L. delicatula* honeydew. I propose an alternative model for understanding the composition of sooty mold colonies, in which the biogeography of all parties (the phloem-feeder, its plant host, and the plant host substrate on which honeydew is deposited), the phyllospheric community prior to disturbance by honeydew, and the all the factors influencing honeydew act as a series of filters which determine a select few sooty mold fungi that will be consistently dominant in a given system. In other words, it is not that sooty mold colony admixtures are entirely random, but that research has not sufficiently defined the factors which determine their composition, or the interactions between those factors.

These findings are very promising in regards to the development of a monitoring tool for *L. delicatula*. The correlation between abundance of *Trichomerium* sequences and sooty mold observations was already cause for optimism, in that in an informal review of phyllospheric literature in North America, *Trichomerium* is not commonly found, and rarely abundant, meaning that our findings seemed particular to the presence of *L. delicatula*. Microscopic observations further confirmed that the *Trichomerium* found in association with *L. delicatula* was of one morphospecies, whose morphological characters and, just as importantly, distribution within sooty mold colonies, was clearly identifiable. These findings alone, with fine-tuning, may be sufficient to inform a monitoring program. However, given the newness of the family, Trichomeriaceae

(Chomnunti et al. 2012), and the fast-evolving nature of mycology and its methods (Hibbett & Taylor 2013), it would be prudent for future work to isolate this *Trichomerium* sp., identify or describe it, and establish its biogeography.

In the early stages of project development, there had been a hope that this research would address the possible impacts of sooty mold to induced plant defenses, and in turn, impacts of sooty mold on plant resistance to *L. delicatula*. The scope of this research was unable to investigate phytohormones, but these findings do suggest that it would be a fruitful avenue of continued research. It may even reveal an opportunity to bolster induced defenses of key plant species to *L. delicatula* and/or its associated sooty mold.

More generally, I find that the ecological approach, the first steps of which were entirely exploratory, was incredibly informative and ultimately applicable. It is not just that there is a moral obligation from a stewardship perspective to examine whole ecologies of bioinvasions, but that these examinations can lead to creative problem-solving in applied research.

I also synthesized existing literature regarding ecologically unequal exchange and biological invasion to theorize how global political-economic dynamics may determine any one nations' vulnerability to the process of biological invasion. I found that the factors known to determine vulnerability to biological invasion were unequally exchanged in the world system, and that bioinvasions themselves are therefore likely also unequally exchanged. Biological invasion fits so neatly into existing ecologically unequal exchange theory, it suggested that biological invasion should *always* be framed as a

socio-economically produced phenomenon. Moreover, the socio-ecological conceptual model did not only better contextualize what is known about biological invasion, but also better scoped out the known and the obscured. In other words, many of the questions as yet unanswered by ecology alone may be addressed by further socio-ecological treatments of biological invasion.

These findings also offered a critique of responses to biological bioinvasions from political, regulatory, and academic actors. The global response to spotted lanternfly may be an example of the missteps that can arise when the socioeconomics of a biological invasion go unaddressed. Within the U.S., for example, impacts to agriculture, especially cultivated grapes received a great deal of early attention research investments, while natural forest and recreational landscapes received a great deal less. It can be argued that this was prompted in part by the reports from South Korea in which grape crops were most affected, but in the years since, it has become clear that spotted lanternfly is likely to be equally or more destructive in natural and recreational landscapes. However, even though timber is a large industry in Pennsylvania, taking into account the region at large, impacts from spotted lanternfly in natural landscapes are poorly captured by economic accounting, as is damage to municipally-owned recreational spaces, especially those with lower-intensity maintenance, where spotted lanternfly is more likely to find *A. altissima*, and untreated plants more broadly. Globally, the countries that are preparing for the entry of *L. delicatula* into their borders, such as France and New Zealand, are those with the resources to do so. However, there are less developed (peripheral and semi-peripheral) countries to which *L. delicatula* also poses a threat (Wakie et al., 2019), which are less likely to have the ability to prevent introduction of spotted lanternfly from the U.S., such

as Argentina, which is one of the top exporters of wine in the world. The full scope of global responses and their effects is yet to be seen. Spotted lanternfly, as well as other, future bioinvasions, should be held up to a socio-ecological lens to test this conceptual model, and to seek better modes of forming local and global responses.

The growing economic and ecological pressure of biological invasions demands not only a swift response, but an elegant one, with solutions aimed not at the symptoms, but the drivers of the issue. To develop these solutions, however, invasion ecology as a field must make great strides in the coming years. Firstly, invasion ecology must turn inwards and develop clearer, more grounded terms to describe their field, and test the operationalization of novel terms and frameworks in theoretical and empirical work. This work has begun in the field and suggests present and urgent opportunities. Secondly, invasion ecology must turn these new frameworks outwards, and integrate theory and hypothesis testing with sister fields previously isolated from invasion ecology.

It is difficult to advocate for a better mode of forming responses to bioinvasions, because innovative work in coalition-building for biological invasions is largely in its infancy and therefore untried. However, some suggestions can be made. A great deal of recent literature in invasion ecology argues that because biological invasion demands prompt and precautionary action, coalitions, funding, and communication plans should be established *a priori*, to enable proactive rather than reactive choice points (Epanchin-Niell et al., 2010; Keller et al., 2011; Peters & Lodge, 2009). My findings suggest that the coalitions built in proactive management plans should include academics from a variety of intellectual backgrounds beyond ecology and related fields, such as sociologists,

political scientists, economists, and should also include stakeholders, and invite community partnerships. Finally, a socio-ecological framing suggest that non-economic impacts should be observed and taken into consideration, including environmental impacts, and impacts to cultural practices and values. Similar coalitions, with similar comprehensive visions have been developed outside of biological invasion and may be useful as models. At Penn State, we can look to “PA in the Balance”, a collaborative project housed in the Agriculture and Environment Center, addressing agricultural land and water conservation which has set goals, and established projects with broad input from agricultural communities (PA In the Balance: White Paper, 2008). Similarly, we might look to Penn State’s Marcellus Center for Outreach and Research, which addresses both current ecological and socio-economic concerns regarding natural gas extraction, and the gradual transition to renewable energy ((Marcellus Center for Outreach and Research, n.d.)

Finally, the conceptual model reveals ways in which imagining new ways of structuring power dynamics to better include the contexts and needs of underdeveloped countries would ecologically benefit not only those countries, but the entire globe, because ecological problems are all ultimately shared problems. These changes, however, would have to occur despite centuries of entrenched power dynamics, including white supremacy and colonialism. With every shared outcome uncovered in this time of ecological collapse, the urgency to untangle these assumptions from our global politics grows, and yet the call has yet to be answered. The ecological problem is existential, it stands to reason that the changes to international relations and policies required to combat

it would be proportionate, but suggestions of radical change bring out apprehension I do not know how to thwart. I add biological invasion to the weighty list of reasons for change, and I hope that the call is answered.

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Appendix A

Chapter 2 Raw Data and Analysis

Raw data and analyses can be found at github.com/mariam-taleb/ITS-Ailanthus-Spotted-Lanternfly.

Appendix B

Chapter 3 Raw Data and Analysis

Raw sequence data and QIIME2 pipeline analyses can be found at github.com/mariam-taleb/Qiime2-Unite-ITSphyllosphere.

Denoised data and R statistical analyses can be found at github.com/mariam-taleb/ITS_Vitis-SLF_Ranalysis.

VITA

Mariam B. Taleb

Mariam B. Taleb was born in Baltimore, Maryland, to Edith Bigelow of Baltimore Maryland, and Haytham Taleb of Al-Tall, rif-Dimashq, Syria. In her youth, she split her summers between those two places and two languages, exploring nature, gardens and farms no matter where she went. This time of her life informed her passion for agriculture, and ecology. She attended school in Baltimore County and graduated high school from the literary arts magnet program at Carver Center for Arts and Technology in 2012.

She attended the University of Massachusetts, Amherst where she double majored in English & in Sustainable Food and Farming, an interdisciplinary agri-food studies program which included studies in agroecology, cropping systems, political-economics of agriculture, and food justice. She graduated magna cum laude with a dual-degree B.A. from her majors as Commonwealth Honors College Scholar with greatest distinction in 2016. After graduating, she worked for the Cornell Cooperative Extension, supporting conventional and organic farms from Rochester to the Southern Tier. This work inspired her to pursue graduate work at Penn State, where she could continue to investigate agri-food studies with an interdisciplinary lens.

Her interests lie in farm sustainability, farm viability, and food sovereignty. She hopes to use her privileges to stand up for others, to lift as she climbs, and to leave her corner of the world better than she found it.