Graduate Research Plan Statement

Do Tropical Lianas Experience Less Disease, Facilitating Conspecific Positive Density Dependence?

Background: Lianas are woody vines that are structural parasites of trees. These vines alter forest community composition, structure, and water dynamics by competing with tree species for light, water, and nutrients and mechanically stressing host trees[1-3]. **Lianas have increased 140%** in Panamanian lowland rainforests altering tree growth and survival, and this trend is consistent throughout the neotropics[1,2,4]. With longer dry seasons and more frequent drought events in the wet season due to climate change, relative liana abundance may continue to increase because of their 63% higher water-use efficiency relative to sympatric trees[1,3]. **Biotic stress from lianas, such as sharing fungal pathogens, which can exacerbate abiotic stress may also play a role but have not been analyzed.** The widely accepted Janzen-Connell hypothesis states that plant pests (i.e. foliar pathogens) cause elevated mortality when conspecific seedlings exist in high densities near parent trees (conspecific negative density dependence, CNDD), thereby freeing resources for the establishment of heterospecifics and higher local diversity[9]. Liana demographic data suggest that abundant lianas do not experience pest-mediated CNDD due to their clonal reproduction of stems and quickly colonizing disturbed sites, instead showing conspecific positive density-dependence (CPDD)[5]. Abundant lianas produce copious amounts of clones near the mainstem, creating this aggregate distribution pattern. Lianas follow traditional ecological theory through highly abundant species having CPDD, but this strategy is usually unsuccessful in the tropics due to pathogen sharing withing dense stands of conspecifics and could result in decreased diversity. Interestingly, the **foliar pathogens of lianas, nor their individual- and forest-scale impacts, have been identified** despite the fact that plant pathogens are believed to be key to maintaining tropical forest tree diversity[9,10].

Objectives: I will collect liana foliar fungal pathogen damage and community composition data to explore potential mechanisms facilitating liana abundance and CPDD. I will focus on fungal foliar pathogens because of the fungal abundance relative to other pathogens, the importance of the photosynthetic capacity for lianas to persist, and leaves acting as a site of pathogen dispersal and exchange. Aim 1 is to connect foliar fungal pathogens to liana abundance and DD patterns. **(H1) Liana species with high abundance resist pathogen mediated CNDD through clonality, population turnover, and colonizing disturbed sites with less disease pressure.** I predict that highly abundant lianas either: 1) exhibit high turnover in clonal stems with pathogen damage, which would result in no correlation between abundance and percent foliar disease; 2) avoid pathogens through chemical defense, which would result in a negative correlation between percent foliar disease and abundance; or 3) maintain higher relative abundances with a stronger CPDD pattern in disturbed versus forested sites, resulting in a positive correlation between percent canopy cover and percent foliar disease. It could be suggested that mutualistic fungi contribute to CPDD patterns; however, lianas have lower arbuscular mycorrhizal fungi colonization than trees[11]. Aim 2 is to determine if disease sharing occurs between lianas and their tree hosts and how this is mediated by abundance. (**H2) Spillover of foliar pathogens occurs between liana and tree hosts through generalist pathogen sharing with varied disease responses.** Either 1) spillover occurs through foliar fungal species being more similar between individual liana and tree host pairs than individual lianas of the same species, or 2) does not occur due to differences between individual liana and tree host. If disease sharing does occur, 3) I expect lianas and trees to have varied percentage of leaf diseased through species-specific immune responses.

Methods: My study will take place in the 50-ha forest plot of Barro Colorado Island (BCI) in Panama, part of the Smithsonian Tropical Research Institute (STRI), where most published data on liana ecology and abundance in the tropics was collected and all lianas >1cm have been identified and mapped[1,2,5-8]. Additionally, a wide variety of long-term environmental and demographic data exist for these forest plots, which can be used to identify explanatory variables and/or control for confounding variables during the study design. **I will sample liana and tree host leaves to identify the percent fungal pathogen damage, fungal community composition, and fungal diversity.** Using 5 species of liana per low (<500 ind), medium (750-1500 ind), and high (>2000 ind) abundance categories from liana demographic data on BCI, 5 diseased leaf samples will be collected randomly from 10 individuals of the same species. The 10 individuals will be sampled from both forested and tree fall gaps to determine the role of disturbance. Paired with each individual liana, 5 proximal diseased leaves from their tree host will be collected. **I will**

measure 1) the percent area of foliar fungal disease for each leaf through scanning and ImageJ analysis and 2) putative fungal pathogen communities will be characterized through culture-based and culture-independent methods. This includes Sanger sequencing of pure strains of fungi isolated from symptomatic tissues and high-throughput Illumina sequencing of the foliar fungal community. I will test whether pathogen damage is correlated with host relative abundance while controlling for other potential predictors, using linear mixed-effects models, and identify important predictors of pathogen damage by comparing models with all or subsets of the fixed effects with the Akaike information criterion[11]. To assess whether the liana and tree species host compositionally unique pathogen communities and pathogen sharing, I will use permutational multivariate analyses of variance, nonmetric multidimensional scaling to visualize compositional differences, the observed number of shared species, and the Morisita-Horn similarity index[11]. To assess specialization, I will calculate the weighted specialization index d′, the extent a species deviates from random host associations, adjusted for host relative abundances[11].

Intellectual Merit: There is a major gap in knowledge in regard to the fungal communities of lianas; there is currently **no published data on the foliar fungal pathogen communities of lianas**. While fungi may play a major role in mediating liana dominance in the tropics, we have virtually no data on the liana mycobiome. My research proposal aims to connect fungal disease extent and community composition with abundance to determine how lianas avoid mortality in highly dense clonal stands. With my **previous research on liana AMF colonization and root endophyte communities**, I have much of the background information needed to understand the current field of liana ecology. As a research assistant for Dr. Erin Spear at STRI, I gained **vast experience in forest disease ecology**. This includes collecting foliar fungal pathogens, scanning leaves for damage surveys, culturing, and DNA analysis. The combination of these research experiences results in my expertise in protocols and background understanding to carry out this research. Additionally, my **strong ties with STRI** contribute to accessible field collection with mentorship and collaboration from prominent scientists in this field. I will be advised by Dr. Stefan Schnitzer, the leading researcher on liana ecology in the tropics, whose research forms the background of this proposal. As a STRI Fellow in Dr. Spear's lab, I will utilize the lab space, instruments, equipment, and chemicals needed for successful completion of this project.

Broader Impacts: Tropical forests are important for the **maintenance of global biodiversity, ecosystem processes, and carbon sequestration**, but are not studied as well as temperate or agricultural systems. With the increasing abundance of lianas, we are seeing a threat to tropical forest community structure and the storage of carbon in plant biomass. Additionally, **forest fungal ecology is vastly understudied**. Working with Dr. Stefan Schnitzer at Marquette University, we will develop a database for information sharing and a standardized methodology for liana demographic surveys in forest plots around the world, housing open-access articles and metadata for use by community, academic, and government researchers. I will lead tours, workshops, and present my scientific findings to the **broader Panamanian community** at the STRI Punta Culebra Nature Center with over 70,000 visitors a year. I will also volunteer on the STRI Q?Bus, a portable classroom and lab **bringing ecological models and experiments to rural K-12 students throughout Panama**. To inspire the next generation of tropical ecologist, I will also volunteer with K-12 schools through the Botanical Society of America's Planting Science Program. This involves **mentoring classroom-based science projects**, instilling a sense of curiosity and interest in tropical forest ecology. This program will reach over 500 students throughout my graduate career.

Sources: [1]Schnitzer et al. 2012. *PLoS ONE*, [2]Martinez-Izquierdo et al. 2016. *J. Ecol.*, [3]De Deurwaerder et al. 2018. *Tree Phys.*, [4]Becknell et al. 2022. *Front. For. Glob. Change.*, [5]Schnitzer 2005. *Am. Nat.*, [6]Ledo & Schnitzer 2014. *J. Ecol*., [7]Schnitzer et al. 2023. *J. Ecol*., [8]DeWalt et al. 2015. *Biotrop.*, [9]Comita et al. 2014. *J. Ecol.*, [10]Spear & Broders 2021. *New Phy.*, [11]Collins et al. 2016. *Oecolog.*, [12]Spear & Mordecai. 2018. *J. Ecol.*