Endophyte effects on plant growth in *Spartina alterniflora*

I. Statement of Work

Introduction

For over 400 million years the success of plants has depended on symbionts (Krings et al. 2007). Endophytes, the symbiotic bacteria and fungi that live inside plants, can help a plant persist in extreme environments (Rodriguez et al. 2009) and mitigate stresses like the high salinity and hypoxic soil found in salt marshes. *Spartina alterniflora*, smooth cordgrass, is a foundational species of salt marshes on the Gulf and East coasts of the United States. The endophyte community of *S. alterniflora* has been shown to shift with stress (Kandalepas et al. 2015), potentially to incorporate or enhance community members that would benefit the growth and survival of the plant host. It is unknown, however, how individual endophytes or groups of endophytes influence *S. alterniflora* growth.

Maintaining and restoring salt marshes in the face of global climate change is essential to preserve the massive ecosystem services of salt marshes, including carbon sequestration, water filtration, and coastal protection (Barbier et al. 2011). Restoration efforts have had mixed results, possibly due to inefficient incorporation of the endophytes into the salt marsh community. I propose to study how the growth of *S. alterniflora* is influenced by individual endophytes and how those effects differ when multiple endophytes are present as a consortium. This will tie into the larger goal of the Van Bael lab to understand the mechanisms through which the plant-microbial community of salt marshes mitigates stress. This will also help to lay the foundation of my doctoral work to examine the endophyte-plant interactions at a genetic level.

Figure 1. Degraded marsh in southern Louisiana. Global climate change is threatening the health of these valuable wetlands. Image by Van Bael Lab.

Background

Recent work in the fields of medicine, agriculture, and ecology has demonstrated the intimate connection between the health and the microbiome of free-living organisms (Christian et al. 2015). A major focus of this research has been endophytes, the bacteria and fungi that live within all plants (Arnold 2007). The interest in endophytes stems, in part, from the positive influence they can have on the host plant's physiology and the desire to harness those qualities for plant management. In general endophytes have been found to enhance a plant's ability to survive in stressful environments (Rodriguez et al. 2008, Friesen et al. 2011). For example, Redman *et al.* (2011) found that introduction of endophytes into commercial rice cultivars increased salt, drought and cold tolerance as well as growth rate of the plants. Song *et al.* (2014) showed that wild barley (*Hordeum brevisubulatum)* was able to better survive in hypoxic waterlogged soil when inhabited by a certain endophyte. Despite these results, there has been little work done on the potential contributions of endophytes to management of coastal wetlands like salt marshes.

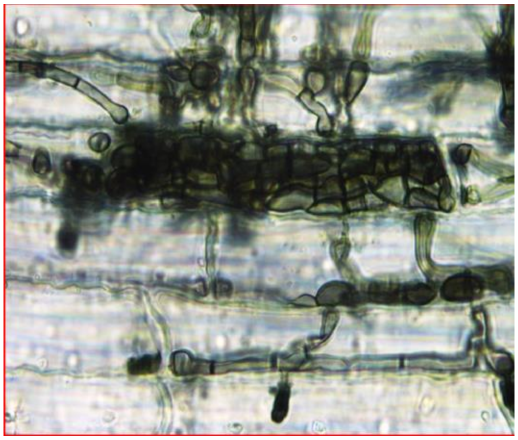

Figure 2. A dark septate endophyte from the roots of Spartina alterniflora. This is an example of one of the many root endophytes in a rich community of microbes that potentially enhance growth of S. alterniflora. Root tissue was cleared, stained and imaged using light microscopy at 100X. Image by Van Bael lab.

Coastal salt marshes are stressful environments subject to increased salinity, inundation and hypoxic soils. Such stressors make it likely that endophytes contribute to the establishment and growth of salt marsh plants. Like many coastal wetlands, salt marshes are economically and ecologically important, providing a myriad of ecosystem services like coastal protection, water filtration, carbon sequestration, and habitat for economically important birds and fish. Unfortunately, many coastal wetlands are being threatened by the effects of global climate change and sea level rise (Erwin 2008).

Efforts to maintain and restore wetlands are expensive and the results can be inconsistent. Because *S. alterniflora* is the foundational species of salt marshes on the Atlantic and Gulf coasts of the United States, restoration efforts of salt marshes have focused on planting *S. alterniflora*. In most cases, the plants are cultivated in greenhouses in commercial soils, so they do not obtain the native salt marsh endophyte community of *S. alterniflora*. Most endophytes are thought to be transmitted horizontally through the soil or air, so there is probably a crucial period in which the endophyte community is incorporated from the salt marsh during which the newly planted grass is vulnerable to stress and less able to compete. However, to my knowledge, no coastal wetland restoration effort has yet to incorporate any endophytes, or the endophyte community of *S. alterniflora,* in management efforts. I propose to develop endophyte inoculants for improvement of restoration efforts and understanding of the salt marsh microbiome.

Although plant-endophyte interactions are complex, we can begin to understand them by examining the effects of individual and simple combinations of endophytes on the plant host. To find endophytes which are useful as tools in salt marsh management and in research we will begin by screening native endophytes. For endophytes to be useful in plant growth promotion, they must not only enhance plant growth, but also be easily cultured for plant inoculations. I have outlined my plans to accomplish this below.

Hypothesis and Anticipated Results

As we move forward in our understanding of the *S. alterniflora* endophyte community, **our hypothesis is that bacterial and fungal endophytes of *S. alterniflora* work individually or in concert to positively influence plant growth.** This hypothesis is based on a growing body of research characterizing the plant growth promoting traits of single and consortia of endophyte inoculants in crop plants (Kim et al. 2012, Mesa et al. 2015, Vandegrift et al. 2015) and preliminary experiments performed in our lab (see part II).

Anticipated Result 1: Plants will grow more quickly and create more biomass in the presence than the absence of individual endophytes.

Anticipated Result 2: Plant growth will be enhanced when plants are inoculated with a consortium of endophytes.

Alternative Result: Endophytes have a negative influence on plant growth. Any effect, positive or negative suggests a strong interaction between the plant and endophyte worth investigating further. This result would be equally interesting, especially if endophyte effects differ depending on whether individual endophytes or consortia are inoculated.

Methods

Seed collection: To obtain a seed bank that reflects the genetic diversity of *S. alterniflora* populations in salt marshes, I visited multiple sites in southern Louisiana in November and December 2015. Seeds were collected by shaking inflorescences into a bucket, so that mature seeds fell from the plant naturally. Seeds were stratified by placing them in deionized water at 4C for approximately 6 weeks. For the proposed work, seeds which have a viable embryo will be selected using a light table, and utilized in the experiments.

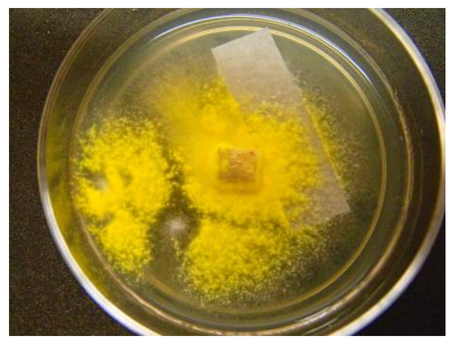

Figure 3. An endophyte isolated and cultured from S. alterniflora. The DNA from isolates like this will be isolated and sequenced for identification. The culture then can be used to inoculate plants to test for growth promoting interactions. Image by Van Bael lab.

Endophyte Isolation: *S. alterniflora* roots will be collected from sites at Bay Jimmy in southern Louisiana and processed within 24 hours. Roots collected from the field will be cut into 5mm segments under aseptic conditions and placed onto 15mm petri dishes containing either 2% malt extract agar to isolate fungi, or nutrient agar to isolate bacteria. Isolates will be grouped by visual characters and DNA extracted via standard methods. Isolates will be identified by Sanger sequencing of nuclear ribosomal Internal Transcribed Spacer (ITS) and 16s rRNA subunit for fungi and bacteria respectively. The two fastest growing bacteria and two fastest growing fungal cultures will be used for the growth chamber experiments.

Growth chamber Experiments: Seeds will be surface sterilized, placed on seed germination paper in a square petri dish and watered with 5mL of water containing ~10^5 CFU/ml of the appropriate endophyte treatment. Gridlines on the petri dish will allow measurement of seedling root and shoot growth. After 2 weeks plants will be transferred to a sterile mixture of sand/hummus (50/50% v/v) in a half-gallon pot. At this time plant tissue samples will be taken and endophytes re-isolated to confirm inoculation. Plants will be allowed to grow for an additional 6 weeks in the growth chamber, then harvested.

Above- and belowground biomass will be compared by drying and weighing plants. Statistical analysis will be conducted in R.

Future Study

Future experiments will build on initial experiments to deconstruct the effects of endophyte constituents upon *S. alterniflora*. In addition to more detailed physiological measurements, I will investigate the genetic pathways and crosstalk between organisms which regulate interactions. Additionally, I plan to study the effects of salinity, inundation, and petroleum treatments upon the plant-endophyte interactions.

II. Completed Work

Because the plants must be endophyte-free before inoculation, they must be grown from surface-sterilized seed. *S. alterniflora* seeds become unviable after 1 season, so they must be collected during the fall for germination in the spring. I collected and stratified seeds from *S. alterniflora* during the fall of 2015. Previous work by our lab has established that there is a rich community of endophytes inhabiting the *S. alterniflora* of Louisiana salt marshes. We also have found that the community shifts in response to stress, potentially to incorporate or increase abundance of endophytes which are beneficial to the mitigation of stress by the plant host (Kandalepas et al. 2015). Preliminary experiments also suggests that *S. alterniflora* grows better in the presence of endophytes than in sterile soil. In testing inoculation techniques it appears that some endophytes may enhance seedling growth as well.

III. How the study benefits coastal wetlands

Spartina alterniflora is the architect of the salt marshes which act as storm buffers, carbon sinks, and water filtration systems throughout the southern and eastern coastal United States. Costly salt marsh preservation and restoration projects have been undertaken with mixed success. Inclusion of properly managed endophyte communities may help reduce cost and improve success of restoration, by improving plant establishment and disturbance mitigation. There is also strong potential for the results to be utilized globally in salt marsh grasses as *Spartina* spp. are foundational to salt marshes throughout the planet.

IV. How funds would be used

Field work ($600): $400 is requested for fuel for the truck and boat to reach the sampling site. An additional $200 is requested for field supplies including wading boots, buckets, supplies for personnel (water, meals, sunscreen, bugspray) **Endophyte isolation ($3020):** $300 is requested for culturing plates, media, ethanol. DNA Isolation: $870 is requested for (4) Qiagen DNeasy Plant Mini Kit. PCR: $750 is requested for primers, buffers, and other PCR supplies. Sequencing: $1100 is requested for sequencing of isolates and confirmation of inoculation. **Growth chamber supplies ($380):** $100 is requested for plant germination paper. $200 for seed germination plates. $80 is requested for sand/humus and plant pots. **Undergraduate Assistant ($1000):** $1000 is requested to hire an undergraduate assistant for 15 weeks. **Total: $5000**

V. Plans for sharing research results with a larger audience

I will disseminate my findings through scientific conferences and academic publications, but most importantly to the public. I am designing an outreach program, tentatively called "Grasses in Classes," and modeled after the successful program of the same name by the Choctawhatchee Basin Alliance in Florida. The goal is to educate and inspire K-12 students of the New Orleans Public School System (in which ~90% of the students are from historically under-represented groups in the sciences) about the important role salt marshes, *S. alterniflora*, and endophytes play in the community. The results of my work will be incorporated into ecological lessons in the classroom and field trips to observe salt marshes and to plant *S. alterniflora.* Our lab also participates in "Girls In STEM at Tulane" (GIST), a biannual workshop for 5th-7th grade girls from New Orleans inner-city schools. Results from these studies will be included in designing the lessons of the workshop. Finally, I will be mentoring an undergraduate student who will work as my assistant.

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