Effect of Soil Microbes on the Growth of Two Santa Monica Mountain Fern Species During Early Gametophyte Development

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Southern California Conferences for Undergraduate Research

Abstract

The fern life cycle alternates between two distinct phases: a diploid sporophyte phase and a haploid gametophyte phase. The mature gametophyte is an independent, single-cell layer structure that is photosynthetic. Much of the research on the early development of gametophytes takes place in sterile conditions while research into the effects of the soil microbiome on gametophyte development has not been well characterized. This study examines the interaction between the soil microbiome and early development of two Santa Monica Mountain fern species, *Woodwardia fimbriata* (Wf) found in riparian streams and *Dryopteris argut*a (Da) which grow in the chaparral understory. Spores from both *W. fimbriata* and *D. arguta* were germinated on agar slides inoculated with water extracts containing microbes from soil samples collected from the base of sporophytes of each of the two species. Over a 20 day period, images of developing gametophytes were captured and gametophyte two-dimensional surface area was analyzed using computational and image analysis software. In the presence of microbes collected from the Wf habitat, *D. arguta* gametophytes display a larger average surface area on Day 18 and Day 20 as compared to sterile and Da soil microbe conditions. In contrast, *W. fimbriata* gametophytes have no significant difference in surface area between the three different soil microbial conditions. Further studies will be undertaken to determine whether the increase in growth observed are due to interactions with specific soil microbes or through some other as yet to be identified factor.

Introduction

The Santa Monica Mountains (SMM), which surround Pepperdine University, are a moderate, Mediterranean-type ecosystem which is characterized by warm, dry summers and mild, rainy winters (Rundler and Tiszler, 2007). While most of the vegetation associated with the SMM are evergreen chaparral shrubs, it is also home to multiple species of ferns (Holmlund et al., 2016). The fern has a sporic life cycle that alternates between two distinct phases: a diploid sporophyte phase consisting of the “adult” fern and a independent, free-living haploid gametophyte phase (Haufler et al, 2016). The mature gametophyte can grow up to a few millimeters in diameter and exists as a single-cell layer structure (Haufler et al, 2016).

The morphological simplicity and external nature of fern gametophytes make them ideal model organisms to study plant growth and development. Studies suggest that the soil microbiome plays an important role in the health of the surrounding plant life (Schnitzer et al. 2011). “Crosstalk” between fern sporophyte roots and soil bacteria has been documented (Bais et al, 2004), while interactions between the soil microbiome and gametophytes has not been as well characterized. Much of the research on early developmental events of fern gametophytes has been carried out in sterile environments which lack the resident microbes of the gametophyte’s natural environment (Ganger et al, 2019). Analysis of the interaction between soil microbes and early gametophytes could provide important insights into adaptation to their environmental niche and their response to environmental changes.

This study seeks to examine the interaction between the soil microbiome and the early development of the gametophytes of two SMM fern species, *Woodwardia fimbriata* (Wf), found in riparian streams, and *Dryopteris arguta* (Da) which grow in the chaparral understory. Since fern gametophytes have been shown to exhibit developmental plasticity in response to experimental conditions (Racusen, 2002), we hypothesized that growing the gametophytes in the presence of soil microbes collected from various habitats in the SMM might have differential effects on the growth of early fern gametophytes as measured by changes in gametophyte surface area.

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Figure 1. Images of SMM fern sporophytes used in study
(A) *Dryopteris arguta*
(B) *Woodwardia fimbriata*

Results

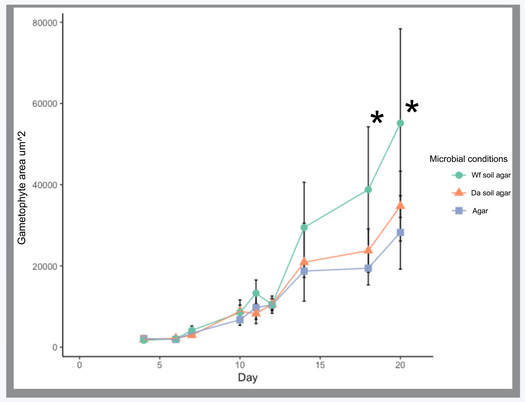

Figure 3. Comparison of the average Da gametophyte surface area over 20 days post-plating between the three media types. Da gametophytes were plated on sterile Bold’s agar slides or agar slides enriched in soil microbes collected from soil at the bases of *Woodwardia fimbriata* (Wf soil) or *Dryopteris arguta* (Da soil). Gametophytes were plated on Day 0 with data points representing averages of gametophyte surface area in the days where growth was present. Asterisks indicate significant difference on log transformed data as shown at α=0.05 as demonstrated by a one-way parametric ANOVA with a Tukey’s HSD test (each point noted with an asterisk has a p-value < 0.05 and F2,14 < 25). Average gametophyte surface area of Da is significantly different in the presence of Wf soil conditions as compared to sterile agar and Df soil conditions on Day 18 and Day 20.

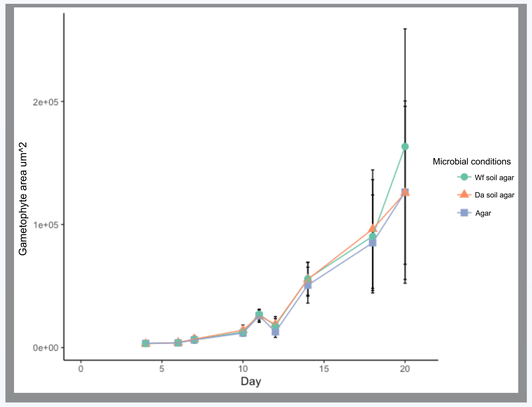

Figure 4. Comparison of the average Wf gametophyte surface area over 20 days post-plating between the three media types Wf gametophytes were plated on sterile Bold’s agar slides or agar slides enriched in soil microbes collected from soil at the bases of *Woodwardia fimbriata* (Wf soil) or *Dryopteris arguta* (Da soil). Gametophytes were plated on Day 0 with data points representing averages of gametophyte surface area in the days where growth was present. There was no significant difference found between the three media types at a α=0.05 as demonstrated by a one-way parametric ANOVA with a Tukey’s HSD test.

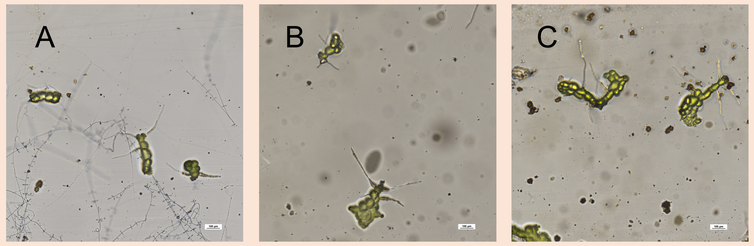

Figure 5. Photographs of Da gametophytes on different soil agar conditions. Da gametophytes were plated on (A) sterile Bold’s agar slides or agar slides enriched in soil microbes collected from soil at the bases of (B) *Dryopteris arguta* (Da soil) or (C) *Woodwardia fimbriata* (Wf soil). Images shown were taken 20 days after plating (100X magnification).

Methods

Collection of Spores
Fronds containing sporangia were collected from sporophytes of two SMM fern species, *Woodwardia fimbriata* (Wf) and *Dryopteris arguta* (Da) in 2021 by Dr. Helen Holmlund. Fronds were placed in glassine envelopes and kept at room temperature to promote dehiscence of sporangia.

Collection of Soil Samples
At 6 different locations at the bases of both *Woodwardia fimbriata* (Wf) and *Dryopteris arguta* (Da) sporophytes found at Upper Cold Creek in the Santa Monica Mountains, approximately 20 g of soil was collected. Soil samples were placed in sterile 15 ml tubes and stored at -80° C freezer until used in analysis.

Preparation of Agar Slides Enriched with Soil Microbes and Germination of Spores:
Water extracts of soil microbes were created by incubating 1g of Wf and Da soil samples in 25 mls of sterile water and incubated on a shaking platform at room temperature overnight. Following removal of soil sediment by centrifugation, the water extract was used to inoculate sterile Bold’s agar. Microscope slides were then coated in Bold’s agar containing either Wf or Da soil microbes, in addition to sterile Bold’s agar as a control. Approximately 25 - 50 Da or Wf spores were plated on slides on each of these three media (Figure 2A). Following plating, samples were placed in humidifying chambers under grow light levels of 25 µmol m-2 s-1 at 100% relative humidity (Figure 2B). Three replicates of each plating were analyzed for each species.

Data Collection and Analysis:
Over a period of 20 days, every two to three days images of developing gametophytes were captured using a Nikon Eclipse Ti2 inverted microscope (Figure 2C) and analyzed using computational and image analysis software (Nikon NIS-Elements platform) in order to quantitatively examine the gametophyte surface area representing the two-dimensional growth of the early gametophyte. In R studio, a one-way ANOVA and Tukey’s HSD test were used at α=0.05 to access significant differences in the traits.

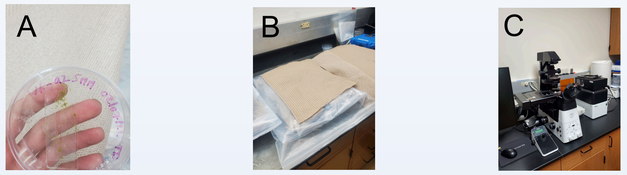

Figure 2. Fern Growth and Imaging Equipment
(A) Petri Dishes
(B) Humidifying Growth Chambers
(C) Ti-2 Inverted Microscope

Discussion and Future Studies

The gametophytes of fern species in the SMM exist in a variety of habitats with different levels of water availability, soil types, and vegetation. The ability of these species to adapt to changing environmental factors could affect the ability of these species to thrive and survive. Analysis of the interaction between soil microbes and early gametophytes could provide important insights into adaptation to their environmental niche and their response to environmental changes. Early events and changes in gametophyte development can have considerable consequences in the morphologies of these different fern species and may facilitate the various adaptive strategies employed by the gametophytes in their different habitats and under changing conditions.

The results of this study suggest that early *Dryopteris arguta* (Da) gametophytes display an increase in growth in the presence of Wf soil microbes as measured by a larger surface area as compared to sterile agar and Da soil microbes. Alternatively, while there was a trend that Wf gametophytes qualitatively displayed a larger surface area in the presence of Wf soil microbes at the end of the 20 day period, statistical analysis revealed no significant difference in surface area in the presence of either soil microbe condition as compared to sterile agar. In both Da and Wf, these differences became apparent at the end of the 20 day growth period, and therefore prolonging the analysis to 40 days may provide more insight into the effect of soil microbes on growth and surface area.

Studies are currently underway in order to identify candidate microbial species for the differential growth effects identified in these gametophytes. 16s targeted amplicon sequencing on bacterial DNA found in both Da and Wf soil samples will be utilized to identify relative abundance of bacterial species in each of the soil samples. If identified, Wf and Da will be cultured in the presence or absence of the candidate microbial species in order to determine the effects of the soil microbes on early gametophyte development. In addition, other factors related to the different soil samples will be investigated to determine whether factors such as soil nutrient availability play a role in the differential impact on early gametophyte growth.

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Acknowledgements

I would like to thank Pepperdine’s Academic Year Undergraduate Research Initiative (AYURI) and the Seaver Dean’s office for funding this research project. Thank you to both Dr. Nofziger and Dr. Holmlund for their invaluable guidance and assistance throughout the course of this research.