Impacts of below ground mutualists arbuscular mycorrhizal bacteria and rhizobia bacteria on above ground mutualistic ants and pollinators across the showy partridge pea (*Chamaeacrista fasciculata*)

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Background

Not a single organism on Earth lives in isolation from their neighbors. These interactions are instrumental in creating the diversity of the ecosystems we see today. Once overlooked, interactions which break down the concept of above ground and below ground communities as separate is a rising area of study. While research in this area has been increasing, questions surrounding above and below ground interactions (specifically between plants and soil microbe interactions) remain some of the most fundamental ecology questions (Sutherland et al. 2013). Plants and their interactions with soil microbes, such as arbuscular mycorrhizal fungi (AMF) and rhizobia bacteria (RH), impact herbivore performance through the alteration of plant nutrients (Bias et al. 2006, Dean et al. 2009, Ossler et al. 2015) and impact plant floral traits which may alter pollination (Barber and Gordon 2015).

The impacts of these below ground mutualists are understudied but extremely important today, as substantial declines in pollinators threaten both agriculture and ecological stability. Current remedies for pollinator losses assume that simply planting more native annual and perennial flowers will bolster shrinking populations. However, community level studies show that additional species beyond flowering plants, including both above ground insects and below ground symbionts, play critical roles in the diversity and stability of pollinator communities (Barber and Gordon 2015).

As a native legume that associates with both RH and AMF, *Chamaeacrista fasciculata* is a fire-adapted annual and bumblebee specialist that grows in grassy and disturbed habitats and is commonly included in U.S, pollinator seed mixes. I previously conducted an investigation at Blandy Experimental Farm demonstrating that RH increased both bumblebee visits and production of extrafloral nectaries (EFNs) which attract ants. However, RH inoculation reduced leaf growth in newly established seedlings, a decrease that might be offset with resources from AMF mutualists. These results were correlations and lacked frequent pollinator or ant visitation during observation periods.

Summary of Proposed Work

To better understand the impacts of the combined mutualisms of AMF and RH on fungal roots, I proposed work investigating the following objectives in either greenhouse or field settings.

1. Investigate the individual and combined impacts of AMF and RH on plant growth.
2. Investigate the individual and combined impacts of AMF and RH on plant pollination through direct changes in plant physiology and indirect impacts on plant health.

3. Investigate the individual and combined impacts of AMF and RH on plant herbivore defense through changes in plant physiology and ant recruitment.

Work Completed

Over the summer, I grew 96 plants which were treated with sterilized soil containing either no mutualists, AMF, RH or both AMF and RH. Once the plants produced their first set of true leaves, I placed them in pots (4in wide, 8in deep) in two field locations: Robins Park, Fort Washington PA and the Dickinson College Farm, Carlisle, PA. Plants were placed in a block pattern design. Once the first plants began to bloom, I collected one plant per soil type per block for root colonization analysis. Carefully, I extracted the roots from the soil and obtained their wet weight. I assessed RH root colonization by measuring the wet weight of any root nodules present. Once plants began to senesce, another set was collected for post-flowering/late-flowering root colonization using the same procedure.

Objective 1 focused on plant growth measurements such as leaf number, time to development stage, plant height, and biomass. I recorded the date of the first set of true leaves, the first EFN and the first flower. Leaf number was recorded during each observation period (about twice a week). After collecting plants for root colonization assessments, I measured the final plant height, leaf number, and EFN number. I also measured above ground wet and dry weight.

Objective 2 focused on pollination. Once plants began flowering, I conducted pollinator observations. This involved observing pollinator visitation for 30 minutes on each plant four times a week. Flower number was recorded for each observation.

For objective 3, I focused on plant defense, specifically through ant recruitment. Each week, I observed the plants for ant recruitment to the EFN per plant in four ten-minute observation periods (Figure 1). I also recorded leaf and EFN count when observations were conducted. Before flowering, I also collected EFN nectar to assess for sugar content.

After assessing the root colonization of the pre-flowering plant group, I continued with ant observations on the remaining potted plants. At the end of the season, these plants were removed from the field and placed in a greenhouse. This followed the same procedures as above.

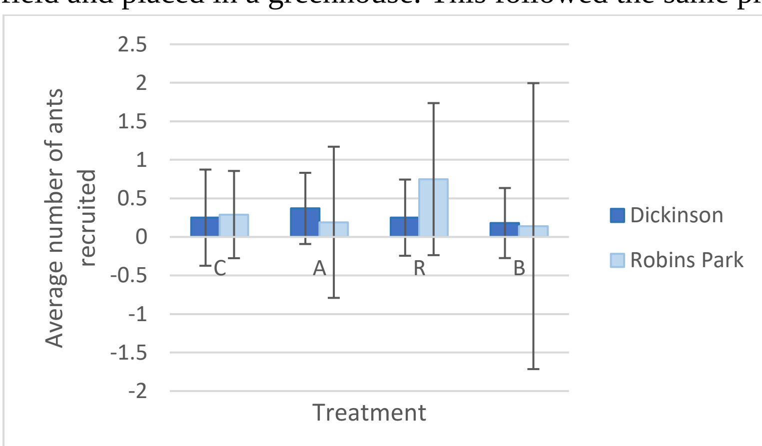

Figure 1. Average observed ant recruitment per treatment at each field site (+/-1 Standard Deviation), before flowering. The treatments are A (AMF), B (AMF and RH), C (no mutualists), and R (RH).

Future Work

Much of the analyses are still being conducted as the lab work continues and a few final measurements are still necessary. Analysis will begin soon on these data investigating mainly RH impacts on the various measurements and plant traits. Unfortunately, during this experiment AMF colonization did not occur. Thus, I am running a greenhouse experiment this winter to obtain non-insect-based measurements of AMF impacts. This experiment will be similar to the experiment conducted over the summer, but the AMF inoculum will be field soil and therefore will contain both AMF and RH; however, the focus will be AMF colonization. I will be measuring AMF impacts on plant leaf, development, height, and biomass. While insect recruitment cannot be directly measured during this experiment, I will be using the following measures to determine impact on pollination: flower number and flower protein content. I will also assess impacts on ant defense by quantifying EFN number and nectar sugar concentrations. Finally, I will conduct feeding trials with grasshoppers to determine herbivore performance.

Once these data are collected, they will be analyzed with the data from this past summer and data from 2018. These three data sets combined will begin to uncover the impacts of AMF and RH both individually and combined (field data 2018), on above ground plant interactions, specifically pollinator and ant recruitment and herbivore preference. This data will be compiled as a section of my master's thesis, a publication, and a presentation at a professional conference in the spring.

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