1. **Objectives/aims** (2 pages)

The resurgence in sustainable farming practices in recent years is driven mostly by interests in improving soil health, nutrient cycling, and carbon sequestration. However, most of the research has focused on utilizing annual cover crops, which are often terminated at the end of the season, and the benefits of alfalfa (*Medicago sativa* L.) in cropping systems have been largely overlooked. Due to its perennial nature, alfalfa can improve soil structure, decrease erosion, and increase carbon sequestration in soil. Increased utilization of alfalfa will not only help to reach ecological goals, but it will also help in improving wildlife habitat and biodiversity, while providing a highly nutritious feedstuff for livestock.

In the United States, alfalfa is the fourth most valued crop behind corn, soybeans, and wheat, with an estimated value of $9 billion (USDA NASS, 2020). Non-dormant alfalfa cultivars are grown in the southeastern United States (Bouton, 2012). In Florida, nondormant cultivars were developed for improved adaptation to the state's subtropical agroecosystem ['Florida 66' (Horner, 1970), 'Florida 77' (Horner and Ruelke, 1981), and 'Florida 99']; however, these cultivars are no longer commercially available due to seed unavailability. Breeding efforts are underway to develop new non-dormant cultivars with improved biomass yield and persistence (Acharya et al., 2020; Biswas et al., 2021). Both yield and persistence are highly influenced by environmental conditions (Figure 1) and understanding genotype by environment interaction (GxE) is critical to develop new cultivars.

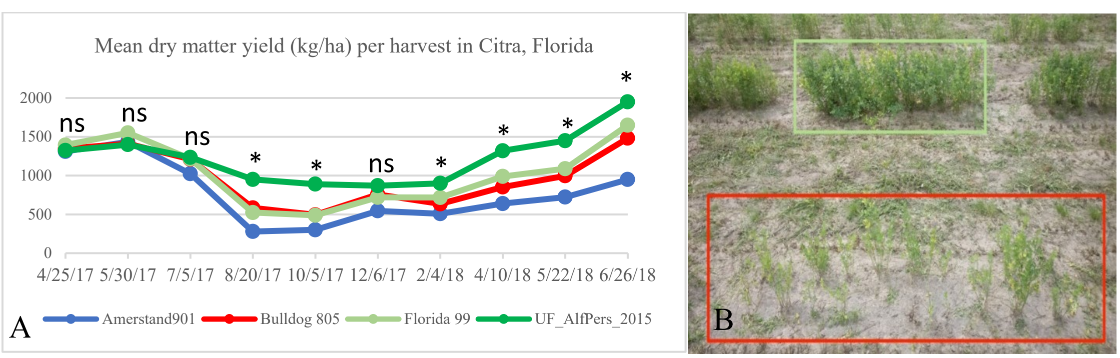

Figure 1. A) Dry matter yield (kg/ha) per harvest in Citra, FL for 4 commercial cultivars across 10 harvests. B) Phenotypic differences for germplasm adapted (green box) and non-adapted (red box) to growing conditions in mid-summer in Florida. Asterisks denote significant differences among cultivars.

Biomass yield peaks in spring and yield declines during summer and early fall in Florida (Figure 1-A). The yield decline is associated with pronounced biotic (disease or pests) and abiotic stresses (heat, water, fertilizer) during summer and early fall, resulting in smaller plants exhibiting lower yield and lower leaf/stem ratio (Figure 1-B). The combination of biotic/abiotic stresses and the reduction in leaf area compromises physiological processes, including biological nitrogen fixation (Ta et al., 1990, Shafique et al., 2014). We hypothesize that the application of nitrogen fertilizer could result in improvements in alfalfa biomass yield and persistence during summer months to compensate for the potential reduction in nitrogen fixation. We plan to expose our breeding population with two contrasting levels of nitrogen inputs (0 vs 30 kg/ha per harvest) to help selection of improved lines that do not need supplemental nitrogen.

Imaging spectroscopy is an established, non-destructive, method for estimating alfalfa yields (Biswas et al., 2021) and monitoring crop nutrient status (Liu et al., 2021). While several studies have estimated nitrogen stress or crop nutrient status using spectral imaging systems (Cilia et al., 2014; Liu et al., 2021; Nigon et al., 2015), methods are not yet available for nutrient stress detection in alfalfa. The utilization of UAVs can speed selection by making available field and plot-scale estimates of foliar nitrogen and biomass and can help boost genomic selection programs (Mir et al., 2019). The main goal of this study is to utilize UAV-based hyperspectral sensors for germplasm screening of non-dormant alfalfa under two contrasting nitrogen levels (0 vs 30 kg/ha per harvest) for biomass yield and persistence in Florida. We will utilize ground-based and airborne sensors for high-throughput phenotyping and genomic prediction models by leveraging genomic resources in alfalfa developed by the breeding insight project. There Bison-Fly pipeline has been created for applications using multispectral sensors, while our project will allow us to create a pipeline for stress detection using hyperspectral sensors. Our study and data analysis will help the breeding community and other plant science researchers interested in plant trait analysis using UAVs. The steps in this project are classified as **(1) Data collection, (2) Image processing, (3) Statistical analysis and interpretation, 4) Pipeline development 5) Workshop arrangement**, **5) Report writing for publication.**

2. **Furthering the aims of the AG2PI** (0.5 pages)

One of the goals of the AG2PI is understanding genotype performance in different environments. This proposal will address the goal number four. This project will involve studying factors that contribute to a decline in yield and persistence in alfalfa under subtropical conditions in Florida. Given the presence of biotic/abiotic stresses in summer in Florida, we hypothesize that biological nitrogen fixation is compromised and results in lower yields and persistence in alfalfa. We propose to use UAVs and non-destructive plant phenotyping methods for nitrogen stress detection and yield/persistence measurements to save labor and time resources. The findings will provide critical information to alfalfa researchers on the impact of nitrogen fertilizer application in summer as to whether it improves alfalfa persistence. Our research will also address another goal (goal 5) of AG2PI; our data analysis pipeline for high throughput phenotyping for abiotic stress (N-stress) will be available publicly, including codes that can be used across multiple crops species. We will incorporate the data collected in this study in genomic prediction models. Our aim is to promote the use of HTP and GS in plant breeding, and we will pursue this goal by making data, data analysis pipeline, and training publicly available. The workshop will target students and researchers in public and academic organizations who are interested in implementing HTP using hyperspectral imagery and GS in their breeding programs.

3. **Expected outcomes & deliverables** (0.5 pages)

Mentoring students and staff in alfalfa breeding, experimental designs, data collection, management and analysis, training activities on implementing HTP and GS in breeding programs, consultation and discussion with breeding companies about implementing HTP and GS in their breeding programs.

Outcomes or Projected Impacts

Change in scientific knowledge on HTP and GS through journal papers, trainings and workshops. Change in action of implementation of HTP and GS in public breeding programs.

Pitfalls that may be Encountered

The projects described here are all typical trials that the PDs are well versed in conducting (Biswas et al., 2021; Rios et al., 2021). All field trials are already successfully established. Therefore, we anticipate that we will be able to make selections as expected and collect data before the end of the grant period. Adverse weather (e.g., a hailstorm or hurricanes) could destroy plots; however, our breeding program has multiple experiments that could serve as a back up.

4. Qualifications of the project team (0.5 pages)

The team of this project is composed of members from different departments at the University of Florida.

Anju Biswas (PI), a Ph.D. candidate, is pursuing her doctorate in three different areas: high throughput phenotyping, genomic selection, and crop modeling. She has experience in high throughput phenotyping using UAVs, (Biswas et al., 2021). She will operate the UAV, process imagery, lead the statistical analysis, write the report and manuscript, create a GitHub repository for data management and analysis pipelines and mentor two undergraduate students.

Dr. Esteban Rios (Mentor), an assistant professor of the Agronomy Department, works on forage breeding applying conventional and novel tools. **Anju Biswas** will lead this project under his supervision. He will also provide other resources to perform data collection in the field, and to genotype the breeding population using the breeding insight array.

Dr. Aditya Singh (Co-PI), an Assistant professor of remote sensing with the Department of Agricultural and Biological Engineering. Dr. Singh's research focuses on the development of remote sensing and high-throughput phenotyping techniques using field-portable spectrometers, UAVs, and airborne hyperspectral imagery. He will mentor **Anju Biswas** in the high-throughput phenotyping and data pipeline development.

6. **Proposal timeline** (0.5 pages) Provide a project timeline with milestones.

Task	June-August (2022)	September-November (2022)	November-December (2022)	January-February (2023)	March (2023)	April-May (2023)
Data collection						
Image data processing						
Statistical analysis and interpretation						
Pipeline development						
Workshop						
Report/Manuscript writing						

6. Engaging AG2P scientific communities & underrepresented groups (Description of how the project will do this; 1-2 sentences)

Two undergraduate students from underrepresented minorities will be recruited form the UF MANNRS chapter to receive training on image processing and analysis, as well as statistical modeling training. The use of HTP and GS is not widely used in forage and turfgrass breeding programs in the USA, therefore, the team will target the training for students and breeders, in public and private programs, for those crops.

Bibliography/References cited

Acharya, J. P., Lopez, Y., Gouveia, B. T., de Bem Oliveira, I., Resende, M. F., Muñoz, P. R., & Rios, E. F. (2020). Breeding alfalfa (Medicago sativa L.) adapted to subtropical agroecosystems. *Agronomy*, *10*(5), 742.

Biswas, A., Andrade, M. H. M. L., Acharya, J. P., de Souza, C. L., Lopez, Y., de Assis, G., … Rios, E. F. (2021). Phenomics-Assisted Selection for Herbage Accumulation in Alfalfa (Medicago sativa L.). *Frontiers in plant science*, *12*, 756768. https://doi.org/10.3389/fpls.2021.756768

Bouton, J. H. (1996). New uses for alfalfa and other" old" forage legumes. *Progress in new crops. ASHS Press, Alexandria, VA*, 251.

Cilia, C., Panigada, C., Rossini, M., Meroni, M., Busetto, L., Amaducci, S., … Colombo, R. (2014). Nitrogen Status Assessment for Variable Rate Fertilization in Maize through Hyperspectral Imagery. *Remote sensing*, *6*(7), 6549–6565. https://doi.org/10.3390/rs6076549

Liu, N., Townsend, P. A., Naber, M. R., Bethke, P. C., Hills, W. B., & Wang, Y. (2021). Hyperspectral imagery to monitor crop nutrient status within and across growing seasons. *Remote Sensing of Environment*, *255*, 112303. https://doi.org/10.1016/j.rse.2021.112303

Nigon, T. J., Mulla, D. J., Rosen, C. J., Cohen, Y., Alchanatis, V., Knight, J., & Rud, R. (2015). Hyperspectral aerial imagery for detecting nitrogen stress in two potato cultivars. *Computers and Electronics in Agriculture*, *112*, 36-46.

Mir, R. R., Reynolds, M., Pinto, F., Khan, M. A., & Bhat, M. A. (2019). High-throughput phenotyping for crop improvement in the genomics era. *Plant Science*, *282*, 60-72.

Rios, E. F., Andrade, M. H., Resende Jr, M. F., Kirst, M., de Resende, M. D., de Almeida Filho, J. E., ... & Munoz, P. (2021). Genomic prediction in family bulks using different traits and cross-validations in pine. *G3*, *11*(9), jkab249.

Shafique, A., Rehman, S., Khan, A., & Kazi, A. G. (2014). Improvement of legume crop production under environmental stresses through biotechnological intervention. In *Emerging technologies and management of crop stress tolerance* (pp. 1-22). Academic Press.

Ta, T. C., MacDowall, F. D. H., & Faris, M. A. (1990). Utilization of carbon and nitrogen reserves of alfalfa roots in supporting N 2-fixation and shoot regrowth. *Plant and Soil*, *127*(2), 231-236

USDA-NASS. (2020). USDA - National Agricultural Statistics Service Available at: www.nass.usda.gov/