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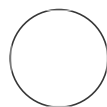
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Forage and Range Research Laboratory Nursery and Plant Measurement Standard Operating Procedures (SOP) and Protocols V.1

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ABSTRACT

The United States Department of Agriculture, Agricultural Research Service, Forage and Range Research Laboratory conducts basic and applied research aimed at improving plant materials and management alternatives for sustainable stewardship of rangelands, pasture, and turf in the western U.S. This research is a multi-disciplinary effort combining expertise from plant breeding and quantitative genetics, molecular biology and genomics, plant physiology, and ecology. This research also relies on standard protocols and procedures to ensure experimental rigor, repeatability, and confidence. This document contains the standard operating procedures and protocols included in the 215 Waldron 2080-21000-018-000D project plan.

Breeding Nursery and Plant Trait Measurements

1 Deep Seeding Emergence (DSE)

- 1.1** Using either Ray Leach cone-tainers SC-10 super cells Place seeds at species-dependent soil depth (5.1, 6.4, or 7.6 cm).
Asay and Johnson (224) reported a positive correlation between emergence rate from a deep planting depth in the greenhouse and seedling vigor on rangelands. Depth of planting in the greenhouse will be 5.1, 6.4, or 7.6 cm depending upon the species. Deep seeding will be accomplished by placing seeds at the given depth into Ray Leach cone-tainer SC-10 super cells (3.8 cm wide X 21.0 cm long) in a greenhouse [22 to 28 °C, relative humidity (RH) = 45%; photosynthetically active radiation (PPFD) = $383 \pm 108 \mu\text{mol m}^{-2} \text{s}^{-1}$]. Or may be done using replicated sand benches, where 50 to 100 pure live seeds (PLS) of each entry will be placed at the bottom of 6.4- or 7.6-cm trenches and covered with a sandy-loam steam sterilized soil. Cones/benches will be watered daily, and rate of emergence day^{-1} determined according to Maguire (225). In some cases, coleoptile length and internode elongation will be measured.

2 Field Plots/Seeded

- 2.1** Seeded plots will range from 1 m x 2 m (irrigated) to 1.5 m x 40 m (semiarid rangelands) and are arranged as a randomized complete block design (RCBD) with 3 to 4 replications unless otherwise specified. Seeded plots are established using either a single-row Wintersteiger cone-seeder or a 5-row Hege precision cone-seeder with a row spacing of 22 cm, at a depth of 0.6 cm and seeding rate of 118 pure live seeds per linear meter of row.

3 Field Plots/Spaced-Plant

- 3.1** Spaced-plant nurseries will be comprised of 5 to 10 plants plot^{-1} and established by hand or mechanically transplanting greenhouse-started seedlings with a spacing of 0.5 m between plants in a plot and 1.0 m between rows of plots. All nurseries are arranged as a randomized complete block design (RCBD) with 3 to 4 replications unless otherwise specified. *Spaced-plant-in-sward nurseries* are a modification to mimic swards by either transplanting into an existing grass or legume sward, or the spaced-plants are over-seeded with such [226].

4 Flowering Date

- 4.1** Record the date (Julian days) on which fifty percent of inflorescences have at least one fully formed flower.

5 Foliar Cover

- 5.1** Estimates of bare ground, weeds and grass canopy cover to calibrate models from UAS imagery will be obtained from close-range RGB imagery approximately one meter from the ground. The software application "SamplePoint" will be used (Booth et al., 2006). Imagery can be captured via smartphones or regular cameras. Rectangular PVC frames (2 ft² size) must be used as reference and will be placed on the ground before taking the camera shots. At least three frames should be used per plot. Annotations of the plot number and picture IDs will be recorded at the field.

6 Forage Mass

- 6.1** Using a sickle bar or flail forage harvester, cut forage plots to an 8-cm stubble height. On research sites unsuitable for the harvester, hand harvest the forage from at least three random 1-m² quadrats throughout each plot. Cut dryland plots once annually and irrigated plots multiple times annually.
- 6.2** Using the onboard or a stand-alone scale, weigh and record the total plot wet weight from each plot.
- 6.3** From the cut forage from each plot, collect an approximately 300 g forage subsample.
- 6.4** Weigh and record the wet, or fresh, weight of each subsample.

- 6.5** In a forced air drier, dry each subsample at 60°C to a constant weight.
- 6.6** Weigh and record the dry weight of each subsample.
- 6.7** Using the following equation, adjust the plot wet weights, to a dry matter basis.
- 6.8** $\text{PlotForage Dry Mass} = [\text{PlotWet Weight} * (\text{SampleDry Weight} / \text{SampleWet Weight})]$

7 Forage Nutritive Value

- 7.1** Using a Thomas Wiley Laboratory Model 4 mill (Arthur H Thomas Co, Swedesboro, NJ, USA), grind dried forage samples (see Forage Mass protocol) to pass through a 1-mm screen.
- 7.2** Place ground sample into near-infrared spectroscopy sample cups.
- 7.3** Using a Foss XDS near-infrared reflectance spectroscopy instrument (Foss, Eden Prairie, MN, USA), scan ground forage samples.
- 7.4** Using the standardized NIRS consortium calibration equations, estimate forage sample values of crude protein (CP; $N \times 6.25$), neutral detergent fiber (aNDF), acid detergent fiber (ADF), acid detergent lignin (ADL), in vitro true digestibility (IVTD), fatty acids (FA), NDF digestibility (NDFD), water soluble carbohydrates (WSC), ash, total digestible nutrients (TDN) (computed

from NDF and NDFD, metabolizable energy (ME) (computed from TDN [219]), and Net energy for gain (NEg) [220] are determined with standardized NIRS calibrations from the NIRS Forage and Feed Testing Consortium.

8 Leaf Area Index (LAI)

- 8.1** Plot LAI field estimates to calibrate models from UAS imagery will be obtained using a Ceptometer. This is an instrument consisting of a data logger and a 90-cm long probe with photosensors that measure photosynthetically active radiation (PAR), which is used to measure light interception, and finally obtain LAI (Parsons et al., 2011). At least five measurements will be taken on each plot - a single above-canopy and 4 measurements below canopy at ground level. Measurements will be taken perpendicular to the direction of the plot.

9 Salinity Tolerance Screening

- 9.1** Seedlings will be grown in a greenhouse under optimal conditions in a 100% silica sand filled cups and watered with a complete nutrient solution until the three-leaf stage. Salt treatments will consist of irrigation every three days by adding a complete nutrient solution with elevated salt (NaCl) concentrations that will be increased every week by an electrical conductivity (EC) of 3 dS m^{-1} until an EC level of 24 dS m^{-1} is reached [222]. Plants will be scored as dead (no green present) or alive (some green available) until 95% of plants within a plot exhibit no green growth. Putative salt tolerant plants are removed following completion of the study and revived using fresh water. Salt imbalance in nutrient solutions is a frequent deficiency in screening studies and cultivar assessments [223]. To avoid an imbalance, NaCl and CaCl_2 will be used in proportions to maintain a sodium adsorption ratio (SAR) of 3.5. The direct EC will be measured with an Orin Model 120 conductivity meter (ThermoElectron Inc., Beverly, MA). When salinity tolerance is evaluated under field conditions, the soil EC will be monitored throughout the study to identify both spatial and temporal variability in soil salinity.

10 Seed Yield and Mass

- 10.1** Measured under spaced-plant conditions by allowing pollination and full seed maturity for each genotype. Seed will be harvested, threshed, cleaned, and weighed. Seed mass will be determined based upon weight of 1000 seeds or may be measured separately by MarviTech seed analyzer.

11 Stand Establishment and Persistence

- 11.1** Seedling or plant frequency will be determined using the grid system described by Vogel and Masters (227) in which the number of 12.5 cm²squares in a 1 m²grid that contain rooted plant(s) of the assigned treatment are counted. This number will be converted to a percentage by dividing it by the total number of squares possible. Data will be collected from each plot during the establishment year, and up to 10 years thereafter, to estimate stand establishment stand persistence. In field sized plots, stand establishment and subsequent plant persistence of target species will be determined using the frequency grid method described above by using 10 grids along a 30 m transects in each plot) and multiplying the frequency of occurrence by a fixed constant of 0.51 to obtain a conservative estimate of plants m⁻¹ (Vogel & Masters, 2001).

12 Turf Digital Image Analysis for Turf Quality

- 12.1** Turf digital image analysis will follow the methods of Karcher and Richardson (218) and use the mobile light/camera box to collect images from each plot on a weekly basis from late April to early September. Digital images will then be analyzed using ImageJ software (imagj.net) and SigmaScan (macro TurfAnalysis.bas, [218]) to determine effect of treatments on ground cover/density, green color, and/or turf quality.

Geospatial Analysis and Modeling of High-Throughput (HTP...

13 Modeling Matrix

- 13.1** Regardless of a classification or regression problem, a matrix composed of m columns (dependent variable and geospatial predictors) and n rows (individual sample observations) will be prepared. Predictors will be the original spectral reflectance bands, derived vegetation indices VIs, digital surface model DSM and topographic derivatives. The values at each column - row ($m \times n$) intersection can be individual pixel values or zonal aggregations (i.e., median, mean).

14 Extraction of Matrix Values

- 14.1** A. In very rare cases, unique pixel values (multispectral, VIs) will be associated with plant traits measured at the field. More often, HTP sample units will be of planar nature (polygons). The boundaries of each sample polygon will be obtained by collecting the corners using GPS rover units or by automatic delineation using the high resolution orthophotos, whichever is more efficient. For each polygon and for each geospatial predictor, all pixel values that intersect the sample polygon will be extracted. Zonal statistics algorithms such as `extract` (Bastin, 2022) that can deal with pixels that are completely contained in a polygon or that can estimate the fractional coverage of a pixel within a polygon will be used. In case raster predictors (VIs, topographic) have different spatial resolutions (pixel size) then the largest grain size will be used for the rest of the analysis, and the rest of the predictor rasters will be resampled to match the largest pixel size.

15 Modeling Schemes

- 15.1** a. non-parametric approaches (support vector machines (Kok et al., 2021), random forests (Cutler et al., 2007)) will be used to optimize predictions accuracies as opposed to model's interpretability (Sheykhmousa et al., 2020). The modeling matrix will be randomly subdivided into training and validation subsets. Model(s) will first be fit using the training subset, and its(their) structure(s) will be simplified to prevent overfitting. Model(s) will then be independently evaluated using the validation subset.

16 Preparation of Spatially Explicit Response Variables

- 16.1** A. once an acceptable error has been achieved with the proposed model structure, then the model will be inputted or applied (Freeman & Frescino, 2018) to the raster predictors included in the chosen model structure. Polygon-level predictions will be obtained for the entire universe of plots for each experiment. This is possible because the UAS imagery will have comprehensive spatial coverage of the plots and experiment.

Plant Breeding and Genetic/Genomic Protocols

17 Plant Breeding Methods

- 17.1** Forage breeding uses recurrent selection methodologies to capture the heritable variation in traits of interest. Methodologies include, restricted recurrent phenotypic selection (*RRPS*), half-sib (HS), half-sib progeny test (*HSPT*), and conventional among-and-within-family selection (*AWFS*) (also called between and within *half-sib family* [*HSF*] selection) [1, 3].

Recurrent selection: Recurrent selection is a cyclical plant breeding process used to increase the frequency of desirable alleles within a population for the trait of interest [1]. A *cycle* of recurrent selection (abbreviated as C_n) for perennial forages consists of four years: one year of establishment, two years of phenotypic and family evaluation, and one year to intermate the selected genotypes in an isolated *polycross nursery*. *Selection intensity (SI)* is the percentage of families or individuals that are selected and polycrossed to complete the cycle. Recurrent selection extends across 5-year project plans, thus the parent plant materials used herein have often been developed in previous projects and are described in detail in Table 1.

18 Genotype-by-sequencing (GBS)

- 18.1 DNA extracted from samples will be genotyped using an established *PstI-MspI* two-enzyme protocol (Poland et al., 2012) and sequenced on an Illumina-based sequencing platform using a licensed core facility. Single-nucleotide polymorphism (SNP) genotypes will be determined from the sequence using the Universal Network-Enabled Analysis Kit (UNEAK) pipeline (Lu et al., 2013) or a reference-based TASSEL GBS (Glaubitz et al., 2014). Genotype assignment for autopolyploid plants will require PolyRAD (Clark et al., 2019) to assign proper dosages. All SNP loci will be filtered so that they align to only one place in the genome, contain a minimum of five (diploid or allopolyploid) or 10 (autopolyploid) read counts per individual to call a homozygous genotype, contain at least two tags with different alleles per individual to call a heterozygous genotype, have less than 70% missing data per locus, are only biallelic markers, and have a minor allele frequency greater than 0.05. This filtering is done in Tassel GBS, PolyRAD, or with custom R and Bash scripts. In addition to filtering loci, individuals with more than 95% missing data are excluded. These marker datasets will be input for genomic selection, diversity analyses, or cultivar/hybrid differentiation.

19 Genome Assembly and Scaffolding

- 19.1 Sequencing libraries from an individual plant are created from high molecular weight genomic DNA and sequenced at third party core facilities with PacBio SMRT cells to produce HiFi reads with at least 20X coverage for each haplotype. Estimates of genome size and heterozygosity are obtained by k-mer analysis using Jellyfish (Marçais and Kingsford 2011) and GenomeScope2 (Ranallo-Benavidez et al. 2020). HiFi reads are assembled using hifiasm (Cheng et al. 2021) or HiCanu (REF). Assembled contigs are scaffolded by proximity ligation using commercial kits (Arima Genomics, Carlsbad, CA) or service providers (Phase Genomics, Seattle WA). Assembly completeness and contiguity are evaluated using assembly metrics from QUAST (Mikheenko et al. 2018) software and surveying single copy orthologous genes using BUSCO (Manni et al. 2021) software, respectively. Scaffolds that are contaminants as identified by Blobtools2 (Challis et al. 2020) and Kraken (Wood et al. 2019), chloroplast or mitochondria as identified by BLAST (Camacho et al. 2009) to the NCBI RefSeq

database, highly repetitive (< 10 Kb non-repetitive sequence) as identified by RepeatModeler (Flynn et al. 2020) and RepeatMasker (<http://www.repeatmasker.org>), or shorter than 10 Kb will be removed to obtain the final assembly.

20 Genome Resequencing

- 20.1** Illumina 2X150 paired-end sequencing libraries will be constructed from genomic DNA of each sample, which will be sequenced at third party core facilities to at least 10X coverage per sample. Sequences will be checked for quality using FastQC(<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>), trimmed using Trimmomatic (Bolger et al., 2014), and aligned to reference genomes using minimap2 (Li 2018). Coverage across the genome will be plotted using the WGScoveragePlotter of jvarkit (Lindenbaum 2015). Presence-absence variants will be identified using a SGSelneloss-based method (Tay Fernandez, et al. 2022). Large-scale structural variants (chromosomal rearrangements, insertions-deletions, duplications) will be identified and annotated using coverage plots and visual inspection of mappings in a genome browser. Single nucleotide polymorphism (SNPs) will be identified from the mapping files of all samples by removing duplicates using MarkDuplicates tool of the Picard toolkit (<https://broadinstitute.github.io/picard/>), then calling SNPs using the mpileup and call functions of bcftools (Danecek et al. 2021). SNPs will be filtered for minor allele frequency > 0.1, 70% max missing data, and minimum quality > 30 using vcftools (Danecek et al. 2011).

21 Genome-wide Association Analysis (GWAS)

- 21.1** Most of the statistical analyses can be completed in the R programming language (Team 2022) using methods published for intermediate wheatgrass (Crain, DeHaan et al. 2021, Crain, Haghighattalab et al. 2021). A mixed model will be used to analyze experimental data and develop BLUPs for each genet using ASReml 4.2 (Gilmour 2021), which are then used as empirical observations for GWAS and genomic prediction. The model fits the inverse genomic relationship matrix, female parent, and units (factor with level for each experimental unit such as plant or half-sib family) with an autoregressive order 1 (AR1 x AR1) model of residual variance to correct for spatial effects. The genomic relationship matrix is created from a mixture of empirical and imputed SNP genotype calls using the A.mat function of the rrBLUPpackage (Endelman 2011) to get the additive relationship matrix. The additive relationship matrix is then inverted from its Choleski decomposition using the chol2inv function, of the R base package. Genotypes used to construct the additive relationship matrix can be replicated so that the size and order of the matrix matches plots or clones that are replicated in the field. The units term is used to fit the 'nugget' variance when a correlation structure, such as AR1 x AR1, is applied to the residual. The Akaike Information Criterion and Bayesian Information Criterion can be used to compare and choose the best model (Isik, Holland et al. 2017). Although BLUPs are predicted for missing observations, only those BLUPs

based on experimental observation are used for GWAS and subsequent prediction and validation of genomic estimated breeding values (GEBVs) described below.

For diploid and allopolyploid populations with disomic inheritance, the GWAS function of the rrBLUP package (Endelman 2011) will be used to perform association analyses using a data frame with SNP markers coded as -1 (homozygote minor allele, frequency > .05), 0 (heterozygote), or 1 (homozygote, major allele) as rows and plants as columns. The additive relationship matrix from the A.mat function is used as K to control the covariance between lines due to a random polygenic effect and principal components determined from an eigenvalue decomposition of the kinship matrix to control for population structure as a fixed effect. Other fixed effects may include different environments of unbalanced experiments. To identify significant associations, a genome-wide false discovery rate (FDR) of 0.05 will be determined using the qvalue function of the qvalue R package (Storey 2002, Storey and Tibshirani 2003).

To make predictions for plants that have not been phenotyped, GEBVs will be estimated by line effects (G-BLUP model) using the kin.blup function of the rrBLUP package (Endelman 2011) with an additive relationship matrix to determine the genetic covariance (G). Here the additive relationship matrix, computed using the A.mat function (Endelman 2011), includes plants from the training population(s) that have been phenotyped and potentially large numbers of plants that have not been phenotyped.

The predictive ability of the G-BLUP models for each trait will be determined by a five-fold cross validation procedure where a unique subset comprising 20% of the BLUPs, based on real experimental observations, are deleted five times so that each BLUP gets deleted once. The five resulting BLUP datasets are used to develop GEBVs for the missing observations (deleted BLUPs). The correlation of GEBVs for missing observations (deleted BLUPs) versus BLUPs based on experimental observations is used to determine the predictive ability of the G-BLUP model for each trait.

The quality and usefulness of a genomic prediction model is only as good as the quality and accessibility of the data used to make the predictions. A key part of any genomic selection program is organizing the data in a manner that is accessible and comprehensible to all users including potential users in the future. Thus, all data used to build genomic prediction models has been and will be stored in a MySQL database (see Data Management) designed from the *Intermediate Wheatgrass* and *Wheat Genetics* database at Kansas State University.

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23 High-density Genotype Imputation

- 23.1** Inexpensive and highly informative genotyping methods are required for breeding and genetic studies requiring a large number of samples. High-density haplotype reference panels of phased genome sequences allow efficient imputations for humans and even some livestock

species, but these resources are not yet available for most plants (Browning and Browning 2013, Browning and Browning 2016, Browning, Zhou et al. 2018, Das, Abecasis et al. 2018, Davies, Kucka et al. 2021). However, relatively new methods for high-density genotype imputation based on extremely low sequence coverage (down to 0.15x coverage), without the need for additional reference panels, are also being developed (Davies, Flint et al. 2016, Ros-Freixedes, Whalen et al. 2020, Whalen, Gorjanc et al. 2020, Whalen and Hickey 2020, Browning, Tian et al. 2021). The Sequencing To Imputation Through Constructing Haplotypes (STITCH) algorithm (Davies, Flint et al. 2016) shows promise for any species with a high-quality reference assembly for read mapping, especially for populations with recent strong bottlenecks that limit the number of founder haplotypes. A pipeline involving standard methods of read mapping, variant calling, variant filtering, and STITCH is being developed and tested for IWG with promising results. The costs are competitive with GBS, but the STITCH imputations produce more than two million high-confidence SNPs per chromosome in Kernza germplasm. The density of SNPs from this approach vastly exceeds that of GBS and provides a powerful approach for breeding and genetic research.

24 Whole Transcriptome Gene Expression Analysis (RNA-seq)

- 24.1** Total RNA will be extracted from individual plants of appropriate tissue types and treatments depending on research objectives. Illumina 2x150 paired-end sequencing libraries will be constructed and sequenced at third party core facilities. At least 20 million sequence reads will be obtained from each sample. Sequences will be checked for quality using FastQC(<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>), trimmed using Trimmomatic(Bolger et al., 2014), and aligned to reference genomes using HISAT2 (Kim et al., 2019). Differential expression analysis will use DEseq2 (Love et al., 2014), focusing on transcripts that are at least 2-fold different between control and treated samples, and with adjusted P-values (FDR) less than 0.05. The resulting DEGs will be clustered based on expression levels in treatment contrasts and visualized using heat maps, a Venn diagram, expression profile charts, and volcano plots. Gene ontologies and functional enrichments of DEGs will be classified using the Trinotatepipeline (Bryant et al., 2017) and Omics Box Blast2GO (BioBam,Valencia, Spain), The WGCNA package of R (Langfelder & Horvath, 2008) will be used to empirically determine co-expression networks among DEGs, based on their expression profiles and visualize the results.

Statistical Analyses

- 25** All studies will be based on generalized linear mixed models analysis. In most cases, our experience shows that errors tend to be normally distributed, allowing us to use normal theory and the reductionist models available in general linear mixed models. In other cases, where errors are not normally distributed, generalized approaches will be used to model proper link functions and distributions of both raw data and errors. Experiments that are repeated in time or space, including sampling dates or times, harvests, seasons, and/or years, will be modeled using various covariance structures to appropriately model correlations among residuals. Larger field trials will

additionally be subjected to spatial analyses to model spatial autocorrelations using various models, to account for the inevitable within-block variability that cannot be accounted by experimental design. Joseph Robins has agreed to serve as statistical consultant for all experiments in this project, drawing upon 20+ years of experience with complex statistical data analysis. Problems that cannot be handled within the group will be referred to the Pacific West Area Statistician.

Unmanned Aerial Systems (UAS) Data Collection and Post-p...

26 Global Positioning System (GPS) Baseline

- 26.1 At each farm / site for which UAS imagery is required, a base station will be established by logging a minimum of four hours of continuous data streaming. These data will be uploaded to the website <https://geodesy.noaa.gov/OPUS/> to obtain a high-accuracy (± 0.02 m) GPS solution for each farm / site. A metal survey marker will be placed at the location of each base station for easy identification in future flights.

27 Flight Mission Planning

- 27.1 Once a spatial domain for the experiment has been mapped, flight missions will be prepared using QGroundControl (<http://qgroundcontrol.com/>) software. Depending on the individual requirements of the experiment and plant trait(s) to be evaluated, a ground sampling distance (GSD) will be defined, and this will be used in conjunction with the multispectral camera specifications to adjust the elevation and speed of the UAS to guarantee a minimum of 75% overlap between camera captures (sidelap and frontlap). If imagery is being collected in non-agricultural settings, then the mission will be planned so that is oriented perpendicular to the general slope of the area. The area to be flown will be at least 10% larger than the area of interest to so that the edges of the fields are completely covered. All missions will be planned using terrain-following protocols.

28 Flight Execution

- 28.1 Flights will be conducted only on sunny days to minimize effects of changing illuminations and will be performed within two hours of local solar noon. Prior to launching the flight mission, a minimum of six highly visible ground control points (GCPs) will be placed throughout the area to be flown. Accurate coordinates of these GCPs will be recorded using a rover GPS that is receiving corrections from the GPS base station via LORA (long range) radio

communications. In addition, images of calibrated reflectance panels (for visible and Near Infrared bands) will be captured immediately before and after every flight. Sensors onboard the UAS will be pointing straight down (nadir or close to nadir). If a particular experiment requires calibrated thermal readings, then three infrared radiometers will be set up to capture continuous readings over three 3 m x 3 m Type 822 fabric airborne sensor calibration targets during the flight duration. These are diffuse hemispherical reflectivity tarps calibrated at 6.5%, 36% and 64% reflectance, representing a dark (hot), medium and white (cold) objects. Temperature readings from these three objects will be used to calibrate the thermal estimations from the UAS Forward Looking InfraRed (FLIR) sensors. Once the flight is finished, imagery files will be copied to a field laptop computer for later transfer to FRR storage and computational servers.

29 Pre-photogrammetry Imagery Post-processing

- 29.1 Individual photos digital numbers DN captured using the Micasense sensors (i.e., Altum, RedEdge) will be converted to reflectance values using the Micasense imagery processing Python scripts (<https://github.com/micasense/imageprocessing>).

30 Photogrammetry Processing

- 30.1 Photos depicting reflectance values will be stitched together using Pix4D or the OpenDroneMap drone mapping software (<https://www.opendronemap.org/webodm/>). Imagery will be georectified using the GCPs coordinates obtained prior to the execution of flight missions. Multispectral reflectance orthophotos, digital surface models (DSM), as well as several vegetation indices (VIs) such as NDVI, NDRE, VARI, RVI will be generated for each flight mission. The file format for the outputs will be GeoTiff. The file naming convention will be Experiment-Farm_Date(YYYY-MM-DD).TIF. **AJH**

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