

Title: Maximizing alfalfa's yield potential

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Abstract:

Alfalfa biomass yield has stagnated in recent decades. At least one possible reason for this stagnation is that breeding programs often do not explicitly measure yield. Further, application of new genomic and phenomic technologies suggests further yield improvements are possible. In this project, we are evaluating UC Davis populations as half-sib families to obtain robust yield estimates and applying both drone-based sensors and genomics to aid selection. We have collected yield data, made selections, conducted intercrosses, and captured sensor data from drone flights on some of the populations. Biomass yield varied significantly among half-sib families and the predicting yield with drones appears feasible at least in some situations. Populations selected based on phenotypes have been developed and genomic selection models will be applied in 2022.

Introduction:

The long-term goal of this project is to demonstrate that genetic gain for alfalfa biomass yield is possible using family-based yield measurements, sensors to estimate yield, and genomic selection to enable marker-only, off-season selection. As a breeding project, this is a long term endeavor, and funding from NAFA was crucial to getting the project started.

Materials and Methods:

We assessed or are assessing three distinct populations for yield based on family evaluations.

(a) Seeded sward vs. spaced plant evaluations of UC2588. In Spring 2017, 105 half-sib families of UC2588, an advanced UC Davis breeding population, were planted in replicated 5-plant plots under full and deficit irrigation treatments in Davis, CA. We evaluated these families for yield and persistence, but by summer 2020, many plants under both treatments had died. Consequently, we evaluated plots for the number of surviving plants and scored each family for vigor based on a 1 = low to 5 = high rating in May 2020. Based on the combined vigor and survival data, we selected 29 plants from 12 families for intercrossing.

In October 2018, 80 half-sib families of the same population that had sufficient seed were directly drilled into 1.5 × 2.5 m plots in Davis, CA. We collected yield and drone-based multispectral data throughout 2019 and 2020. Based on the yield data, we selected the top 12 families for intercrossing.

(b) Transplanted half-sib family rows under saline and non-saline irrigation. We transplanted 65 half-sib families of UC-Impalo into 8 plant replicated plots under saline and non-saline irrigation in May 2019 at the Westside Research and Extension Center in Five Points, CA. We measured

yield monthly and noted the number of surviving plants during late summer and fall in 2019 and throughout the year in 2020. Based on high yielding families under both saline and non-saline conditions, we selected 33 surviving plants that were vigorous and free from root rot diseases from the saline plots in autumn 2020 and intercrossed them in the greenhouse.

(c) Half-sib family transplanted sward evaluations. In May 2020, we transplanted 200 half-sib families derived from UC5976 and UC2705, two elite UC Davis breeding germplasms into 24-plant plots in two replications at two locations in Davis, CA. UC5976 was derived from selections made in nurseries of UC2705 and several related UC breeding populations, and thus, the families are all closely related. We had limited seed of each family, so we transplanted plots with 24 closely spaced plants (Fig. 1). We planted one trial in Yolo County (Davis) and the other in Solano County south of Davis. We had hoped for a location in Fresno county in the San Joaquin Valley, but due to Covid-19 restrictions at the time of planting, this wasn't possible. The two sites are rather different in terms of soil type and inherent fertility.



Figure 1. Left and right – one replication at each of the two sites the UC-5976 and UC-2705 half-sib families were planted in Yolo (left) and Solano (right) counties in Northern California, 2020. Center – the transplanting arrangement of the 24 plants per family, necessitated due to limited seed availability.

We measured yield for three harvests in 2020 and have taken seven harvests in 2021. We have taken samples from the April 2021 harvest for forage quality evaluation. In addition, we have multispectral sensor data from drone flights at each harvest, and for two harvests, we have hyperspectral data from a fixed 50 ft. pole imaging the entire plot areas.

Project Objectives and Corresponding Results:

Project Objectives:

1. Determine genotypes and evaluate yield of half-sib families derived from multiple University of California breeding populations.
2. Use drone-based sensors to develop robust predictive

Project Results:

1. We have identified individual plants and half-sib families with high biomass yield to form new experimental cultivars. We will compare populations selected for vigor from spaced plant trials with those selected for yield from sward trials. We obtained DNA marker data for two populations will use them for marker-only selection in early 2022.
2. We have collected sensor data using multispectral and hyperspectral cameras in 2019, 2020, and 2021. The results show that in certain harvests, sensor data is very

equations for biomass yield based on row and sward plots.

useful for predicting yield. In other harvests, especially in spring harvests in the first year, when all plots are still looking very good or when yield variation among families is small, the relationship between drone data and actual yield is moderate. We see utility in predicting yields in some harvests, for example, by measuring one replication with a harvester and predicting other replications without taking yield measurements. This could expand the number of families or populations we could evaluate at a given time.

Results and Discussion:

Objective 1. Determine genotypes and evaluate yield of half-sib families derived from multiple University of California breeding populations.

(a) UC2588 half-sib families: Based on yield data from sward evaluations across 2019 and 2020, we identified the top 12 families (15%). As can be seen in the figure below, a number of families had superior yields compared to the check cultivar Impalo in 2020. We selected the best individual plants from within those families to develop an experimental population based on yield data.

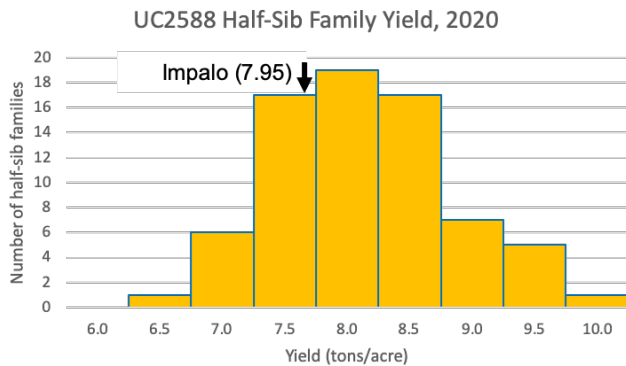


Figure 2. The distribution in total biomass yield across all harvests in 2020 of half-sib families from UC2588 compared with the check cultivar 'Impalo.'

In the separate, spaced-planted trial, we selected 29 plants from the top 12 families to develop an experimental population based on spaced plant vigor and survival. Interestingly, only one of the 12 families was in common between the two evaluation trials. One reason for the discrepancy is that we were able to evaluate more families as spaced plants than in seeded swards, because some families did not have sufficient seed to direct drill, and some of these families, tested only in spaced plants, were selected. In other cases, though,

families that performed well as spaced plant did not perform well in swards. Thus, these two populations will explicitly test our initial hypothesis that yield improvement requires evaluation for yield in sward plots and not for vigor/persistence as spaced plants.

We will compare the selections from the spaced-planted and sward trials with the original population to determine if we have made progress for yield or other traits in trials planted in 2022.

(b) Impalo half-sib families. We selected plants based on yield measurements of families grown in saline and non-saline irrigation at the Westside REC in Fresno County. We identified families that had high yield under both conditions, and then selected plants from those families in the saline irrigation treatment that had good vigor and disease-free roots and shoots. Under saline irrigation, many plants had died, imposing a considerable selection pressure. We selected 33 plants (11 in each replication) from the saline plots and intercrossed them in the greenhouse. The resulting population will be compared to Impalo, the source of the families, in trials to be seeded in March 2022 to determine if we made progress for yield under saline or non-saline irrigation.



Figure 3. Left: a plant selected under saline irrigation, showing the severe mortality imposed by saline irrigation. Right: one replication of the saline and non-saline treatments for evaluating half-sib families of UC-Impalo.

(c) UC2705 and UC5976 half-sib families: We harvested the half-sib families of our advanced breeding populations seven times in 2021, the first full production year. The yield data show a large range in productivity among the families (Fig. 4). Many families have yields higher than their source population. The yield of UC5976 seems unexpectedly low; we might expect it to be toward the mean of the families. It could be that the plants that produced sufficient seed for the trial are on average better than the overall population, and this could account for the result. Alternatively, the UC5976 plots may simply have performed poorly for other reasons. In any case, a wide range of family performance is present, enabling selection for higher yield.

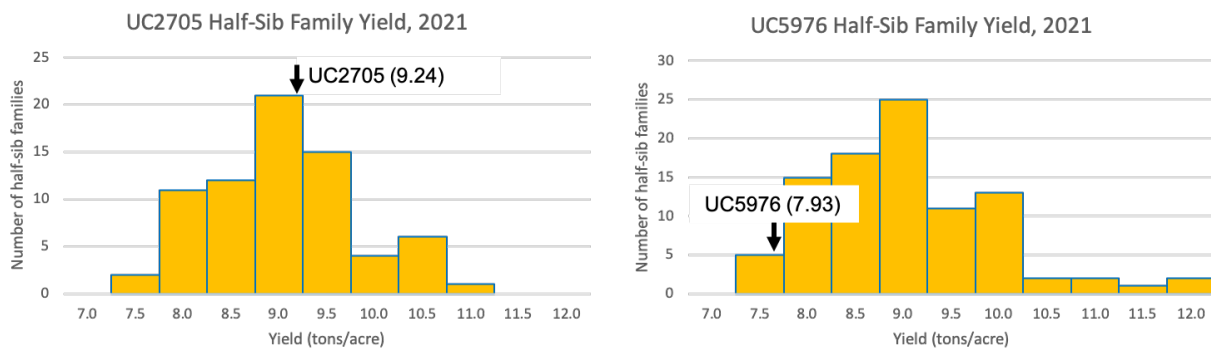


Figure 4. The variation in total biomass yield across all harvests in 2021 for two UC breeding populations. In both cases, many families with yield higher than the base populations are present.

Leaf tissue has been collected in bulked samples for all families and DNA has been extracted. We used genotyping-by-sequencing, based on our past protocols, to generate ~20,000 high quality, genome-wide SNP (single nucleotide polymorphism) markers on this population. Using those markers, we assessed the population structure of these two breeding populations (Fig. 5). The two UC breeding populations share similar parentage, with UC5976 being developed from parents selected from UC2705 as well as several other UC populations, all of which are related to one another and to the cultivar UC Impalo.

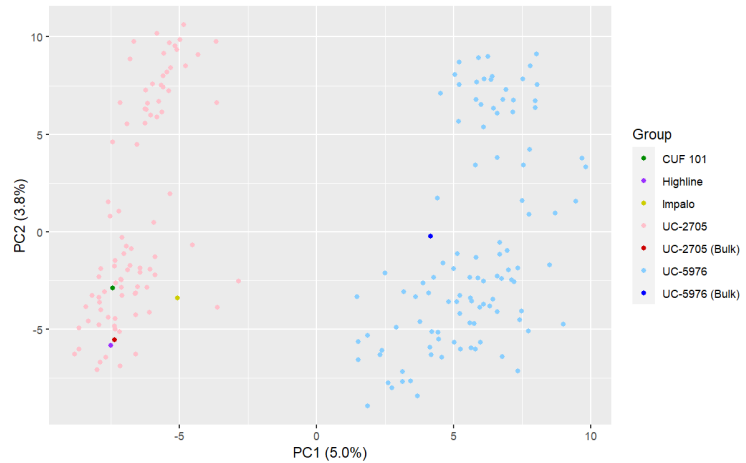


Figure 5. A principal components analysis (PCA) of half-sib families derived from two UC breeding populations, UC2705 and UC5976, the base populations (as indicated by “bulk”), and three check cultivars.

Interestingly, the UC2705 and UC5976 families cluster in separate groups, each with their respective base population. The UC2705 families cluster with the three check cultivars all previously developed in the University of California breeding program. But interestingly, UC5976 appears distinct from them. This is a useful result, actually, because it will enable us to determine if a genomic selection model can be developed to predict yield across closely related, but still genetically distinguishable, populations. To be useful in actual breeding programs, this type of flexibility will be needed, and we can test that in this program as we move forward.

Objective 2. Use drone-based sensors to develop robust predictive equations for biomass yield based on row and sward plots.

We had generated sensor and yield data for the UC-2588 sward trials in 2019, and those results were quite compelling (Fig. 6). These data suggested that predicted yields based on the sensor data could give very good fit to the actually measured yield data. Were these numbers to hold up, we could possibly skip collecting yield data at some harvest dates. Two important criteria for

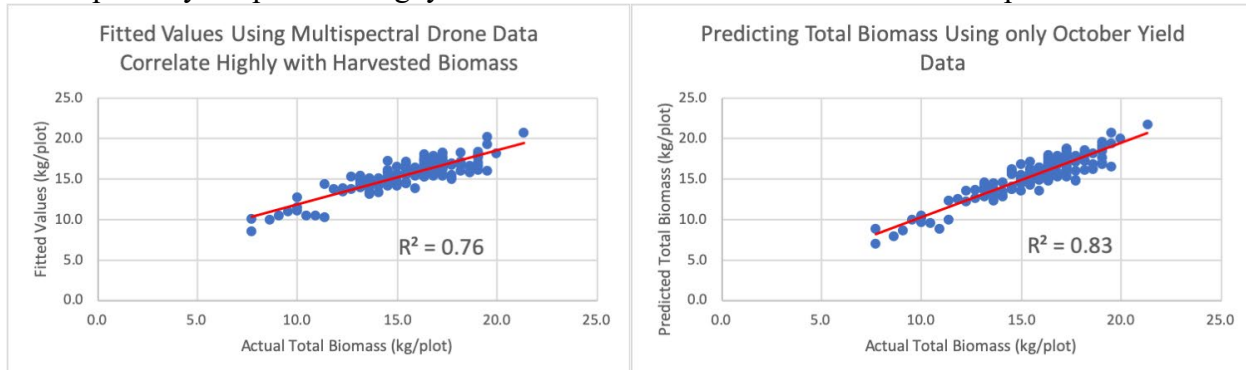


Figure 6. The relationship between actual measured yields and drone-based yield predictions based on plot volume measured on the UC2588 half-sib family sward plots.

computing plot volume from drone imagery are an accurate discrimination among plots and an ability to reference the ground level at each plot location. The way we had planted this trial, using closely spaced sward plots with no gap between them turned out to be a major impediment to getting good drone information, necessitating hand clipping between plots and keeping plot alleys clipped and weed-free prior to each harvest.

For the 2020 half-sib trials, we transplanted families at high density unlike the direct seeded trials for UC2588 above. Across all harvests in 2021, the R^2 between measured yield to sensor-based biomass prediction was 0.49 (Fig. 7), which is not high enough for routine use in a breeding program. However, the relationship between actual yield and predicted yield varied considerably across harvests, and the relationship in August 2021 at the Yolo County location was 0.85. Thus, drone-based sensor data will be more useful in some harvests than others, and this will dictate how best to integrate them into a breeding program.

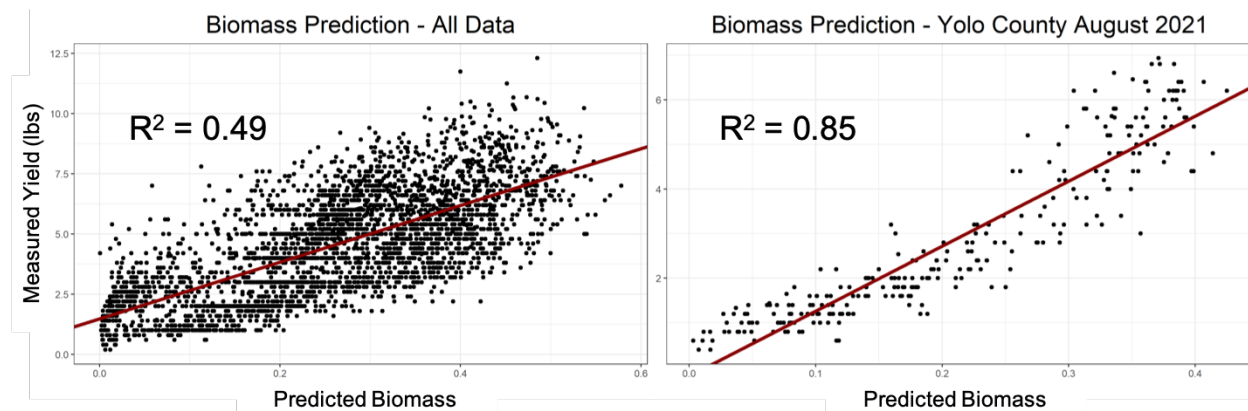


Figure 7. The relationship between actual measured yields and drone-based yield predictions in UC2705 and UC5976 half-sib families grown in Yolo County, CA in 2021.

These trials provided easy measurement of soil levels in alleyways, but differentiating the outer range of plots from seeded borders has interfered with our accuracy. In a few cases, we harvested borders prior to flying the drone, and in these harvests, our accuracies improved markedly. Thus, as mentioned above, we need to reconsider how we layout trials, what plot arrangements we have, and how we incorporate open alleyways for soil baseline determination. Thus, efficiency gains from using phenomic technologies, as well as genomic tools, can probably be realized more routinely if we re-envision the overall program rather than simply applying them to our current programs using standard plot methods that have worked in the past.

In cases where the relationships between yield harvest and drone estimation are the greatest, we could envision several ways to use drones. Most obviously, we could harvest only part of a trial – perhaps one replication and use the resulting yield-sensor relationship to predict the yield in other replications. Early in a trial, most family plots tend to be very robust and show little obvious variation; this is reflected in less discriminatory sensor data. The sensor data appears to be most useful in later harvests and later years of a trial, when biotic and abiotic stresses have begun to lead to plant mortality and differential vigor among plants. Under these conditions, using a yield-sensor data relationship from one harvest can be applied to a subsequent harvest or harvests, to further decrease the number of plots that need to be measured with the harvester. Further opportunities to use drone data will undoubtedly arise as we get more proficient with their use.

Conclusions

We have shown that yield estimates vary widely among half-sib families of four different University of California breeding populations and that this information can drive selection for improved yield. While we need to wait for evaluation of new populations to show if we have made progress, the variation we observe is certainly encouraging. We have applied drone based sensors to estimate yield, and the data here are also encouraging. If we are able to predict yield, at least in some cases, we could save considerable time compared with mechanically harvesting all plots across all harvests. Finally, we have genotyped two of these populations to generate genomic selection models to (hopefully) further increase efficiency for increasing yield.

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