Title: DEVELOPING HIGH YIELDING AND HIGH QUALITY ALFALFA VARIETIES AND CROPPING SYSTEMS FOR HIGH SALINITY CONDITIONS

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ABSTRACT

Salinity is a major challenge for alfalfa growers in irrigated regions and even in many non-irrigated regions. There is a necessity for varieties highly tolerant of salinity, and for production methods to grow alfalfa under saline conditions. Field trials were conducted in a cooperative project between the University of California, Davis and California State University, Fresno. Two field trials were conducted in western Fresno County (a saline region), utilizing specialized reservoirs and injection of highly saline water directly to alfalfa root systems utilizing subsurface drip irrigation systems combined with surface waters. The objectives were to 1) test the relative performance of alfalfa cultivars under high salinity in a replicated trial; 2) determine the economic viability of alfalfa crop production under high-saline conditions, and 3) to develop new germplasm and varieties utilizing screening plots at high salinity. High salinity (HS) irrigation levels (ECw of 8-11 dS/m) were compared with low saline (LS) controls (<1.2 dS/m ECw) utilizing a split plot design. Yields averaged 89% of Low Saline plots over 2014-2017 in Trial 1. In Trial 2, yields averaged 95% and 78% of non-saline controls in 2017 and 2018, respectfully, indicating the buildup of salinity effects

over time. Over the two years of study the V x S interaction was non-significant. Absolute yield level under HS shows a relationship to relative yield (% of control, $R^2 = 43.6\%$), indicating the value of understanding absolute yields as well as relative yields. Alfalfa yields under HS were economically viable for this region (average yield under HS = 9.6 t/a), indicating a high level of salinity tolerance for alfalfa in general, which directly contradicts textbook values which predicts alfalfa yield decline at low (EC=2.0) salinity. These trials will be continued through 2019 and 2020, since long-term impacts of salinity are important.

INTRODUCTION

Nearly 50% of US alfalfa is produced in the western region mostly under irrigation. In many areas, saline conditions dominate, either in water supply or soil characteristics. Often, 'degraded' water (from municipal sources, irrigation drainage, saline wells, animal facilities, or food processing facilities) is more available than fresh water for irrigation. Furthermore, so-called 'higher value' crops (vegetables, orchards, vines, specialty crops) are likely to be grown on the best land and available water, competing with alfalfa and grain crops, a trend to be exacerbated in the future. Cropping systems and varieties adapted to a more saline future are needed.

More broadly, salinization associated with irrigation has been estimated to impact as much as 20 percent of the world's irrigated farmland (Munns, 2011). In alfalfa-growing regions, high salinity waters and soils can be found in southern Canada, northern Plains states (ND, SD), the Pacific Northwest states of WA, OR, and ID, the southern plains states of TX, OK, NM, AZ, the high desert regions of the Intermountain West (UT, NV, CO) and in the desert Southwest (AZ, NM, CA). Salinity is particularly a problem in irrigated regions. Salinity threatens the long-term sustainability of agriculture in the San Joaquin valley (CA), with millions of acres of good soils rendered unproductive due to drought and salinity (Schoups et al., 2005).

Alfalfa is one of the more profitable agronomic crops in many areas, and is <u>the</u> key crop for western dairies – which now account for about 48-50% of US milk produced. A wide range of water sources are impacted by salinity (e.g. Colorado River, irrigation drainage areas, processing wastes, municipal and livestock wastewater) and could be used with appropriate management to produce alfalfa. Future cropping systems for saline areas are not likely to include the higher-value vegetable crops, or orchards/vineyard crops, which are generally highly salt sensitive. Forages and grains will continue to be moved onto more marginal land and irrigated with lower-quality water as high-value vegetable, fruit, and nut crops utilize the better soils and water sources. Additionally, salinity tolerance results in poor water uptake, it may be highly related to drought tolerance, an important objective for the future.

Thus, there are considerable incentives to develop alfalfa varieties and management systems for saline conditions. This research was undertaken to 1) test the performance of alfalfa cultivars in replicated trials; 2) determine the economic viability of alfalfa crop production under high-saline conditions, and 3) to provide the infrastructure in the field for breeders to develop new germplasm and varieties utilizing screening plots at high salinity.

METHODS AND MATERIALS.

These field trials are a part of a longer-term cooperative program with University of California (Davis) and California State University (Fresno) to understand the interactions of alfalfa with salinity, in cooperation with seed companies and plant breeders to develop new cultivars adapted to saline conditions. These have included greenhouse and field studies previously conducted. We have had 3 students from Fresno State University and 1 student from UC Davis (funded separately from this project) on this project, and we have featured these research results at field days and at state-wide meetings to farmers.

Field trials were conducted at the UC West Side Research and Extension Center, Five Points, CA. PI's had developed much (but not all) of the infrastructure necessary to conduct salinity work at this site, including 3 reservoirs (200' x 60'), holding tanks, a mixing area for adding salts, mixing pumps, pipes for delivery, and installation of drip tapes.

UC Advanced Variety Yield and Quality Comparison Trials. There were two trials conducted during this period to compare variety performance under high and low salinity levels.

Trial 1. Yield Trial (2014-2017) High- and Low- salinity plots were planted in the fall of 2014 and harvested in 2015 and 2016, and in 2017. Plots were planted in two separate basins (Low Salinity, LS and High Salinity, HS) with a forage planter at 36" width and 15 foot long plots. Irrigation water was applied via flood irrigation to the LS and HS sections. Approximately 7 harvests were measured each year. Water applications and salinity levels were monitored and quantified.

Trial 2. Yield Trial (2017-2020) was planted in the spring of 2017, and was irrigated with both saline and non-saline water in different treatments. This is a split plot design with four replications (Figure 1), with salinity as a main plot and 35 varieties as sub-plots. Each plot measured 36" x 15 feet length. Yield data was collected over 7-8 cuttings per year. Selected plots analyzed for forage quality and mineral (Na, K) uptake.

Trial 3. Breeding and Germplasm Development (screening basins). We have established 8 basins for this purpose, utilizing transplants or seeded plants, enabling the development of high-yielding alfalfa cultivars tolerant of saline irrigation water. These are primarily semi-dormant and non-dormant varieties adapted to the Southwestern US and desert regions around the world. These screening trials were made available to public-sector and private sector breeders. Our goal was to identify genotypes that are vigorous and highly productive under the stress conditions.

Water Management: An Integrated Irrigation/Surface Irrigation System. We developed an infrastructure to deliver high saline water to test plots. Water was delivered both through subsurface drip lines and surface irrigation systems to increase distribution uniformity and control the leaching fraction. Specialized reservoirs and pumps to add salts to water and mix to a specific EC have been developed. Trials 1 and 2 were irrigated with high saline water 7 to 12 dS/m – resulting in soil ECs of 10-16 dS/m over the life of the screening plots. Salts (combination of NaCl and $CaCl_2$) were added as needed to obtain the desired salinity level of 9-11 dS/m ECw (ambient saline water from this specific well was approximately 6.0-7.5 ECw). This practice is compared with 'normal' (low saline) irrigation water of EC approximately 1-1.5 dS/m, replicated in separate basins. In Trial 1, water was applied via flood irrigation in small basins. The higher salinity (HS) irrigation waters were applied in Trial 2 via a combination of subsurface drip irrigation and flood irrigation to mitigate some of the soil structure and subsurface water supply problems that are due to salinity.

Our aim in Trial 2 was to reduce the variation in root zone salinity with depth associated with soil drying cycles and inadequate water applications to provide a leaching fraction. The trial included salt-tolerant and susceptible control varieties, plus plots for experimental and commercial lines. Salinity irrigation treatments and yield monitoring began in 2017, continued through 2018, and will be completed in 2020.

Trials were integrated with extension activities via field days and grower meetings, and linkages with breeding and irrigation companies were developed. We made presentations of this data at the California Alfalfa Symposium in Reno, NV in November, 2018, and at the NAAIC meeting in Salt Lake City in 2018, and at the World Alfalfa Conference in Argentina in November, 2018.

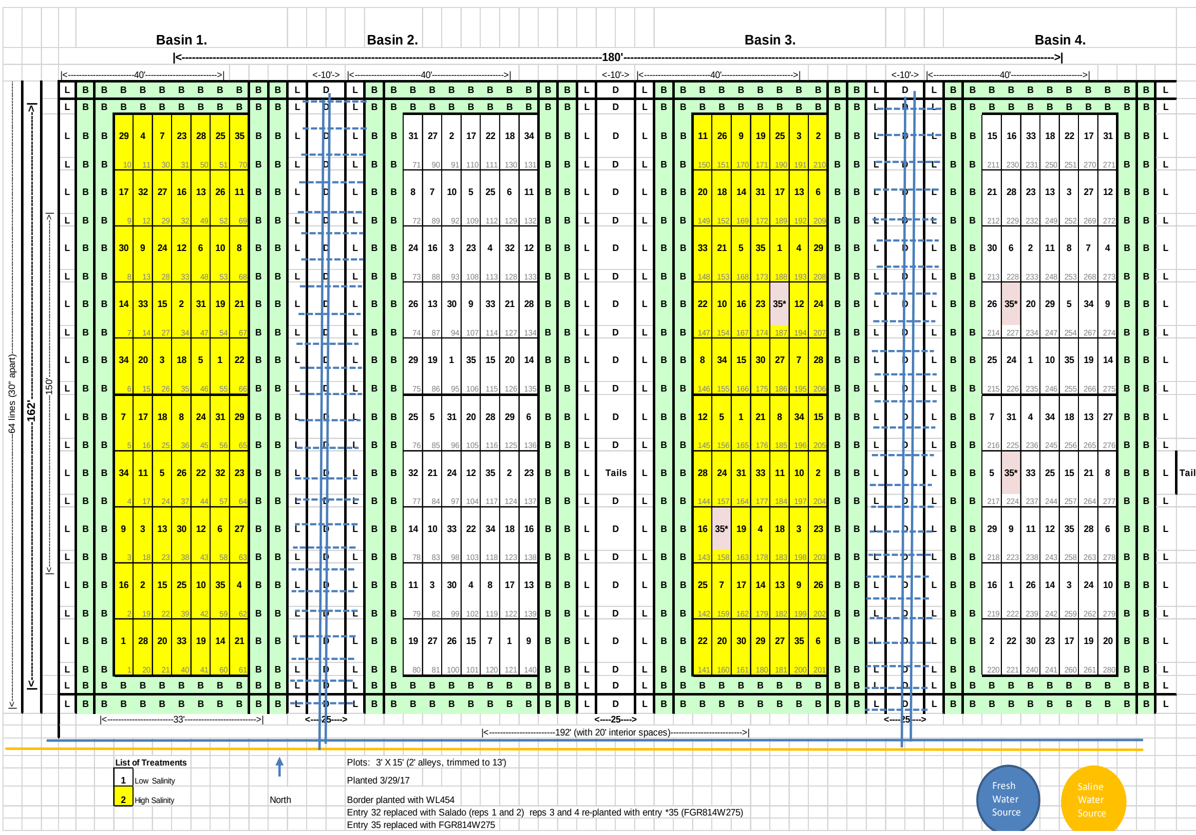

Figure 1. Layout of the 2017 UC Salinity Advanced Yield/Quality trial (trial 2). A combination of drip irrigation and flood irrigation was used to improve distribution uniformity. Drip lines (12" deep) run perpendicular to the field plots. Double borders are used to reduce border effects.

Data Collected):

- Yield response to salinity (2 trials: approximately 3,000 yield observations/year, corrected for dry matter), 7 cuts per year.

- 3-4 harvests/year from selected varieties were taken to determine forage quality (NIRS).
- Mineral uptake of selected samples as budget allows for Na+ or K+ concentrations.
- Gravimetric soil moisture data over the season at 5 depths (beginning and end of season)
- Monitoring of soil moisture and EC by depth via sensors (Teros 12 sensors).
- Aerial photography of growth spatial patterns to ascertain whether spatial growth patterns as caused by soil variation which can be used as a covariate to improve variety comparisons. Reflectance data can be utilized to detect differences in salinity tolerance between varieties.

Analysis of Shoot sodium as a selection criterion for salt tolerance. There are moderate correlations between Na+ concentrations in alfalfa shoots and salinity tolerance (r^2 values of 0.65). Samples were taken to examine whether sodium exclusion and possible interaction with potassium (K+/Na+ratio), can be used as a possible indicator of salinity tolerance. Samples were taken during 2017-18 to test these hypotheses and are still being analyzed.

Spatial Analysis to account for soil variability. We have conducted spatial analysis with overhead drones and periodic photography to identify and quantify growth patterns for observed spatial patterns. Since soil variability has been a major impediment to field variety evaluation, this could be a very valuable tool. The objective here is to characterize stress spatially in the trial as well as to remove extraneous sources of variation that may ultimately interfere with our ability to measure cultivar differences due to salinity. This information can be utilized to develop an understanding of alfalfa stand and plant growth parameters as they are affected by salinity stress.

RESULTS AND DISCUSSION

This is a long-term effort to understand the salinity-variety interactions, which began in 2009 and these trials are anticipated to be completed in 2020. Alfalfa is a perennial, and both short-term yield and quality impacts as well as long-term impacts on stand persistence under highly saline conditions are needed to understand these effects. Two trials were funded in this period:

Trial 1. This trial was planted in 2014 and completed in 2017. Soil salinity was impacted by the treatments over the life of the trial (Figure 2). Applications of saline water increased soil salinity significantly in year one and salinity increased over the 3 years of the study in the top three soil depths (0-90 cm). In the HS basin, soil salinity increased by 95% during the first year of saline irrigation (2015), going from 5.9 in May to 11.5 dS/m in October for the 0-90 cm depth. In the second year (2016), soil salinity increased by 55% in response to HS irrigation, going from 10.6 in May to 16.5 dS/m in October for the 0-90 cm soil depth. As in LS, there was a slight decrease in soil salinity in the 2017 final soil sampling date, with ECe going to 15.9 dS/m.

Yields in Trial 1 were significantly impacted by salinity (Figure 3). Average yield impact of salinity across varieties was 11% in this study over three years, but ranged from 13% to 9% from year 1 to year 3. Strangely, salinity affected alfalfa 3rd year yields less than in year 1, even though there was a buildup of salinity over this period. Varieties differed significantly in overall yield in each year and over-the-years, but the variety x salinity interaction was not significant. Although we had careful management of salinity applications in these basins, the variation within basis was sufficiently high that specific variety responses to salinity could not be confidently made. This was a source of frustration, and led to our implementation of SDI combined with surface water applications to achieve more uniform supply of water in the root zone (Trial 2).

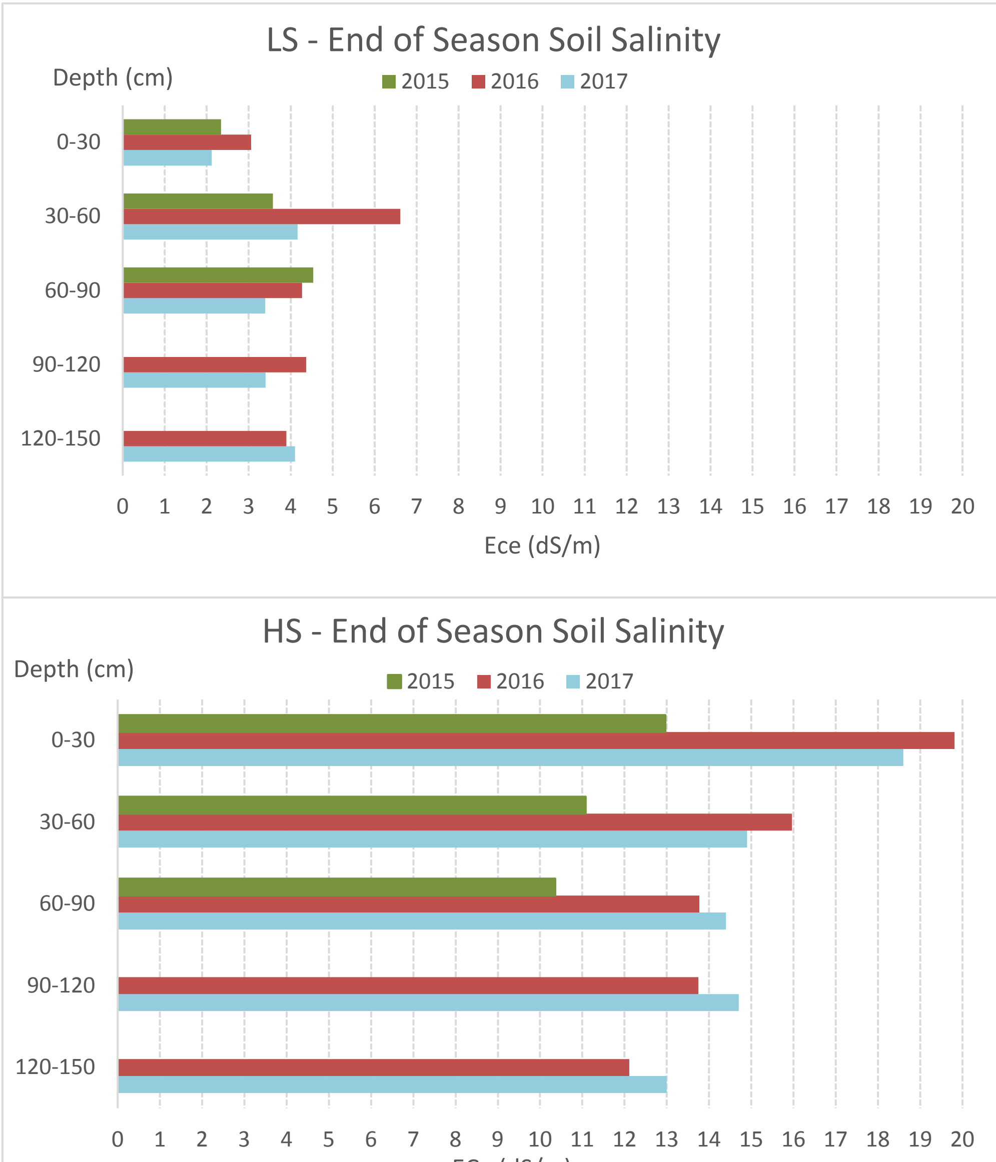

Figure 2. Distribution of soil salinity (ECe) in the soil profile in HS basin (Trial 1). Samples were collected in 3 depths (0-30, 30-60, 60-90) in 2015, and 5 depths (0-30, 30-60, 60-90, 90-120, 120-150 cm) in 2016 and 2017.

Sodium/Potassium uptake. Under saline conditions, Na is favored over K, and as are both monovalent ions they are thought to compete for uptake within the plant. Thus the K/Na ratio tends to be lower in the high salinity plots and is associated with lower shoot dry matter yields (Figure 4). However, this relationship is not particularly strong (R^2 = 40%).

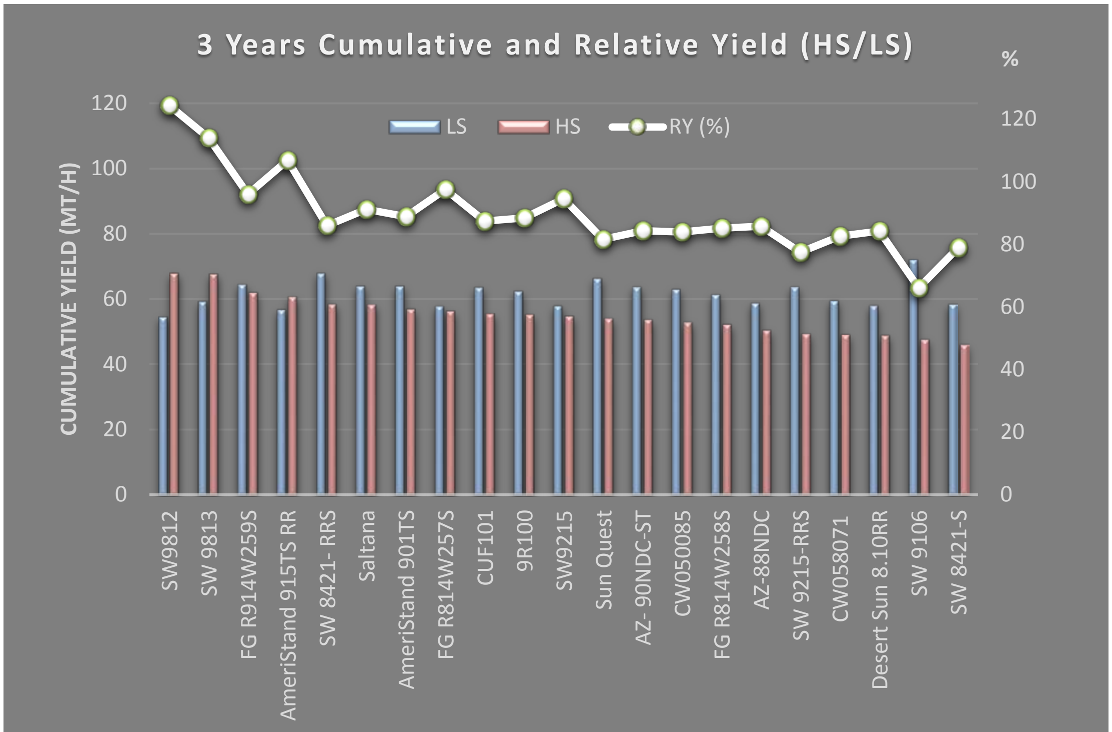

Figure 3. Cumulative Yield (sum of 2015, 2016, and 2017, dry matter basis) in Trial 1 and relative yield percentage (HS/LS*100) of 21 alfalfa varieties grown under high saline (HS) and low saline (LS) conditions, western Fresno County, CA. Approximately 7 harvests per year. Varieties are arranged in order of cumulative yield under HS condition.

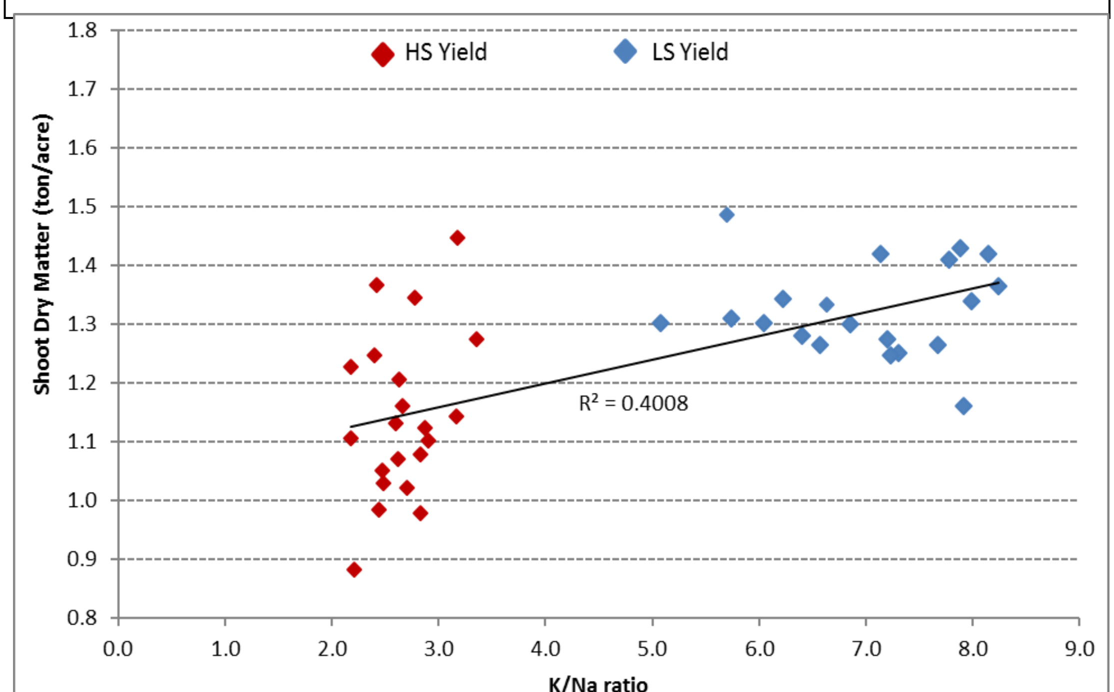

Figure 4. Relationship between K/Na ratio and shoot dry matter (Trial 1).

Trial 2. This trial was planted in the spring of 2017 and harvested in 2017 and 2018, and the trial is anticipated to be continued through 2019 and 2020 (funding permitting). In this field trial, we took a slightly different approach than in Trial 1, and implemented a subsurface drip irrigation (SDI) system to improve the distribution uniformity (DU) of water within each of the basins (LS and HS). Similar target salinity levels were implemented as in Trial 1. We used a combination of SDI and surface irrigation (Sprinklers) to allow sufficient leaching and distribution of both water and salts within the root zone. Soils and water were monitored (data not shown), and the ongoing salinization of the root zone analyzed.

Yields in this trial were measured in 2017 – 2018 with only 5 cuttings in 2017, but 7 cuttings in 2018, which is typical of spring-planted alfalfa in this environment. Each harvest plot yields were measured and plant samples taken for dry matter determination and subsequent analysis for mineral uptake and forage quality (some of this data is pending further analysis). Yields were affected by salinity an average of 4% in year 1 and 22% in year 2, indicating a buildup of salinity across the first two years of study (Table 1).

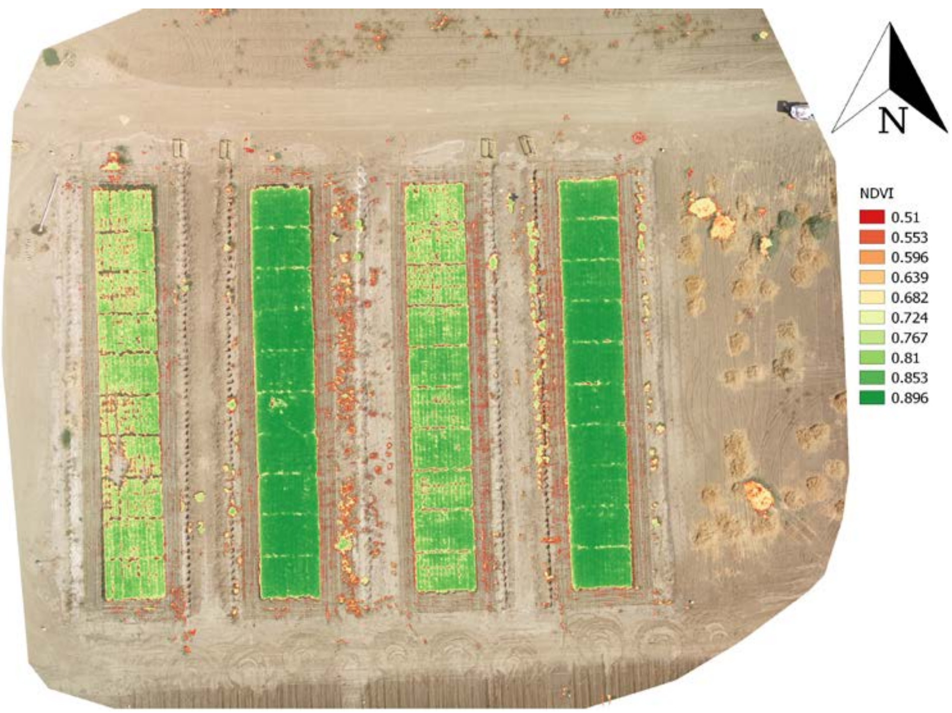

Figure 5. Drone image utilizing multi-spectral camera (MicaSense Red Edge M) utilized to calculate NDVI. Saline basin (1st from left and3rd from left) can be clearly seen, and the individual varieties discerned. Image was taken August 15, 2018 at an altitude of 30 m with 85% front overlap (Trial 2). Additional images were taking using thermal cameras.

Imaging of the Trial. In Trial 2 we were able to take a series of aerial images utilizing both thermal and multi-spectral cameras utilizing drones during the growth of the crop in 2018. An example of one of our images is provided in Figure 5, which clearly indicates the stress differences between the saline and non-saline plots. A total of 8-10 flights were taken in 2018, during growth periods and just before each harvest during summer months. The purpose of this was 1) to detect stress in the varieties caused by salinity, and 2) to attempt to account for field variability and its impact on yield and variety comparison. We have correlations between yields and image pixel density, and our aim is to develop those relationships to assist in analysis of field trials.

Relative Yields. In many of the studies on salinity, it is common to calculate the 'Relative Yield' (RY) of a variety in relationship to its yield under non-saline or low-saline conditions. This calculation is putatively related to a 'salinity tolerance' characteristic or genes related to salinity tolerance. This calculation is common in both published greenhouse and sand-tank studies, although it's important to point out that growers do not harvest 'relative yields', but absolute yields. In our studies, relative yields (Yield under Saline conditions/Yield under non-saline conditions) were generally related to yield under saline conditions (Figure 3, Table 1). However the linear relationship (Figure 6) was not strong (R2 = 0.4).

Implications: Economic and Agronomic Viability of Alfalfa Grown under Saline Conditions. This research, accompanied by earlier greenhouse and sand-tank work, indicate that alfalfa is much more saline tolerant than previously reported, and can be grown with acceptable yield levels under more saline conditions (with careful management) under field conditions. FAO and other reference documents peg alfalfa as being saline tolerant only up to EC of about 2.0 dS/m, and yields subsequently decline about 7% for each additional unit increase in salinity. These calculations would project a yield decline of 50% or greater at the salinity levels we examined. These projections were largely developed utilizing sand-tank studies where alfalfa lines were subject to frequent watering. However, in our trials, salinity caused yield declines of 11% (Trial 1) and 17% (Trial 2) in the field. Additionally, the absolute yield levels under high salinity (HS) in our trials averaged 8.1 tons/acre in trial 1 over 3 years of study, and 9.6 tons/acre in the second full production year in Trial 2, yield levels that are quite acceptable economic yields for the West Side of the San Joaquin Valley, where average county yields are approximately 8.5 t/acre. These indicate a high level of alfalfa tolerance to saline conditions. Perhaps more important than the average response of alfalfa to salinity is 1) the effect of high saline waters on water infiltration and water availability, and 2) the increased variability induced by salinity, 3) the long-term effect of high saline waters on the buildup of salts and sustainable soil management. The intensified soil variability we have observed in salinity trials is a challenge to both plant breeders and agronomists, and is difficult to manage. We've observed spatial variation in soil salinity of double or even triple the EC levels within meters within our fields. Our approach of using a combination of subsurface drip irrigation and surface irrigation to move salts down in the profile has been modestly successful (so far). Since long-term effects of salinity are so important, our objectives remain to continue these trials until 2020 to test variety response to salinity and measure long-term effects of salinity on crop response to yield and quality.

ACKNOWLDGEMENTS

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Table 1. Westside Alfalfa Salinity Trial yields . Varieties were grown under low (EC_w of 1.0 to 1.3 dS/m) and high saline (ECw of 7.0 to 10.5 dS/m) conditions utilizing saline well water with added NaCl and CaCl. Relative yields (HS as a percent of LS) are shown. (trial planted 3/29/17 at the University of California West Side Research and Extension Center, Five Points, CA).

	2017 Season Yield (ton/A)		2018 Season Yield (ton/A)		Cummulative Average (t/A)		Relative Yield
Variety	**Low Salinity**	**High Salinity**	**Low Salinity**	**High Salinity**	**Low Salinity**	**High Salinity**	**%**
UC Salton	5.2	5.2	12.0	12.2	**17.2**	**17.4**	100.9%
SW8421RRS	5.0	5.5	13.5	11.3	**18.5**	**16.8**	90.8%
SW9573	5.5	5.1	12.0	11.3	**17.6**	**16.5**	93.6%
9R100	5.5	5.0	14.6	11.1	**20.1**	**16.2**	80.6%
SW9106M	4.7	5.5	12.9	10.4	**17.6**	**15.9**	90.5%
Integra 8810S	4.9	4.6	12.9	11.3	**17.8**	**15.9**	89.2%
AZ-88NDC	6.0	4.7	13.7	10.9	**19.7**	**15.7**	79.7%
C0916ST232	5.3	4.8	12.9	10.7	**18.3**	**15.5**	84.7%
H0715ST209	4.5	5.0	10.2	10.4	**14.8**	**15.4**	104.2%
H0916ST223	4.5	4.9	10.8	10.5	**15.4**	**15.4**	100.0%
SW9215RRS	4.8	5.2	13.3	10.1	**18.1**	**15.3**	84.4%
SW9577	5.2	5.0	13.7	10.2	**18.9**	**15.2**	80.5%
CUF101	4.9	4.6	12.9	10.4	**17.7**	**15.0**	84.7%
UC Impalo	4.5	4.9	12.7	9.9	**17.2**	**14.8**	85.8%
PGI 908-S	5.4	5.0	14.3	9.8	**19.8**	**14.8**	74.8%
H0916ST218	4.9	4.9	12.1	9.4	**16.9**	**14.4**	84.8%
SW9576	4.9	4.6	11.5	9.5	**16.4**	**14.1**	86.0%
R814W257S	5.2	4.6	13.7	9.4	**19.0**	**14.0**	74.0%
SW8409	4.9	5.0	11.7	9.0	**16.7**	**13.9**	83.7%
SW8476	4.5	4.8	13.1	9.1	**17.7**	**13.9**	78.7%
R814W258S	4.8	4.7	11.8	9.1	**16.6**	**13.8**	83.2%
AZ-90NDC-ST	4.8	4.4	12.5	9.3	**17.3**	**13.8**	79.9%
R914W259S	4.2	4.5	11.5	9.0	**15.7**	**13.5**	86.1%
H0716ST227	4.2	4.4	10.6	9.0	**14.9**	**13.4**	90.2%
H0915ST214	4.3	4.0	11.3	8.9	**15.6**	**13.0**	83.0%
H0916ST217	4.0	4.2	10.4	8.6	**14.3**	**12.8**	89.4%
FGR814W275	3.5	3.6	10.5	9.2	**14.0**	**12.8**	91.1%
H0715ST211	5.0	4.0	11.5	8.6	**16.5**	**12.6**	76.4%
H0815ST210	4.7	4.0	11.8	8.4	**16.5**	**12.5**	75.7%
H0915ST212	4.8	3.9	11.1	8.4	**15.9**	**12.3**	77.3%
AFX149092	4.7	4.0	13.5	8.3	**18.2**	**12.3**	67.5%
H0716ST222	4.9	4.3	12.9	7.9	**17.8**	**12.2**	68.9%
H0916ST216	4.5	3.7	11.5	8.5	**15.9**	**12.2**	76.7%
SW8412	4.9	3.7	12.3	8.4	**17.2**	**12.1**	70.2%
Salado*	4.4	3.2	13.3	8.1	**17.7**	**11.3**	64.2%
Average	4.8	**4.6**	**12.3**	**9.6**	**17.1**	**14.2**	82.9%
Yield loss	**4%**		**22%**		**17%**		
CV%	**16.30%**		**16.50**				
LSD (p=o.o5)	**0.18**		**1.80**				
treatment	*		**				
variety	***		***				
treatment X variety	**ns**		**ns**				

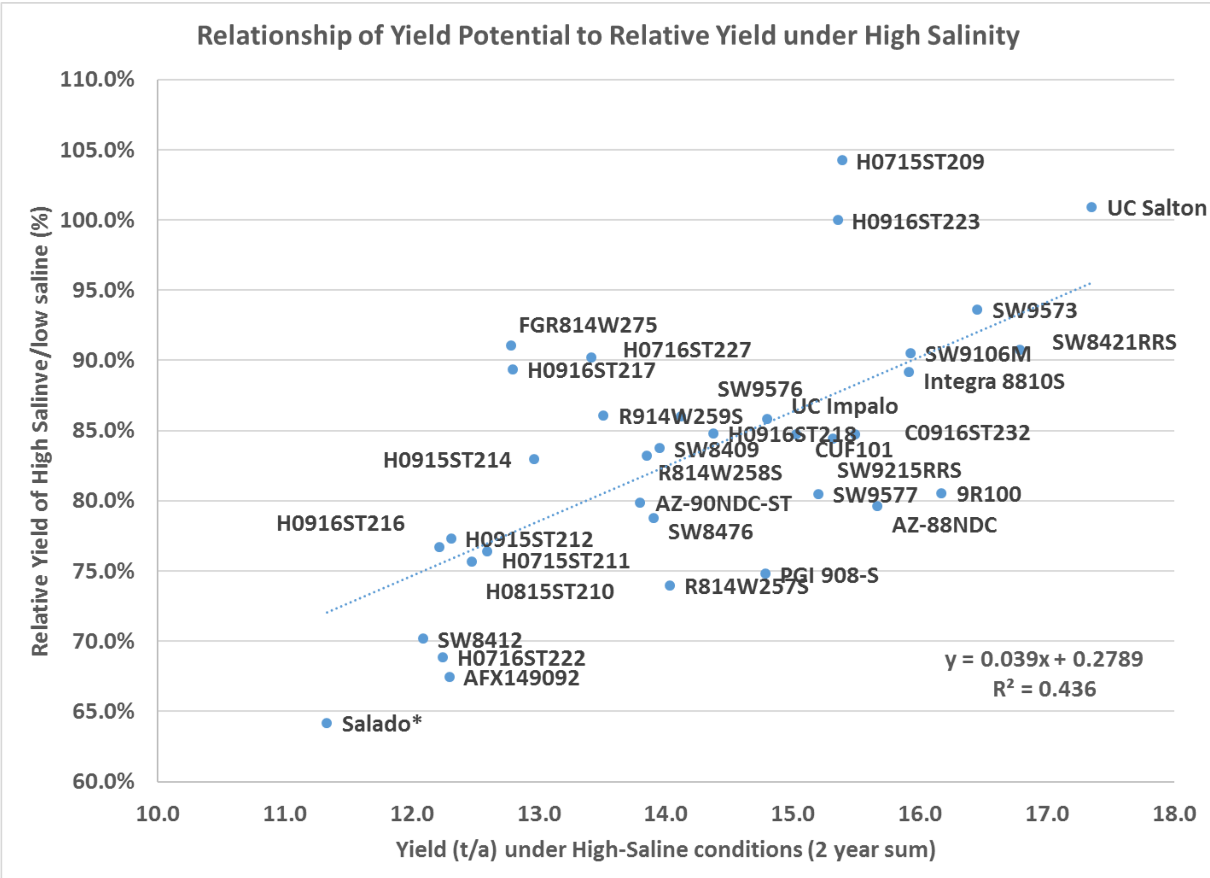

Figure 6. Relationship of yield under high saline conditions to the relative yield of each variety (Trial 2). Relative yield is the ratio of yield under High Salinity divided by low salinity yields.

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