Improved Methods for Nutrient Tissue Testing in Alfalfa

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3. CDFA Funding Request Amount/Other Funding.
Total funding requested from CDFA is $59,886 for the remaining years of this project. There is no additional funding available for this project, as there is not commodity board for alfalfa research. However, we are able to take advantage of the subsidized analysis costs provided by UC Division of Agriculture and Natural Resources Analytical Laboratory, located in UC Davis, which significantly lowers the cost of the project. Additionally, we have funded 3 additional years of sample collection with our own funds previous to the FREP grant – and this grant will be the beneficiary of that data.
B. EXECUTIVE SUMMARY

Alfalfa is the most critical feed for the state’s #1 agricultural enterprise, dairy. It occupies between 930,000 acres and 1.1 million acres, and thus represents a very important component of California’s fertilizer and agricultural footprint—primarily due to a high requirement for phosphorus and potassium. Due to the high acreage, nutrient management of this crop has potentially large impact on the agro-ecosystem of the state.

Analytical methods have been developed to assess the nutritional status of alfalfa fields for fertilizer management purposes. Soil tests are somewhat effective to detect some nutrient deficiencies but plant tissue tests are believed to be far more accurate overall. Unfortunately, most alfalfa growers do not tissue test and growers fertilize (or don’t fertilize) based upon past practice with little idea of the actual nutrient status of the field. Additionally, tissue testing techniques vary significantly from state-to-state. Simplified methods of analysis could promote wider adoption of nutrient monitoring practices and perhaps encourage the adoption of standardized methods between regions or states.

Many alfalfa crops in California are routinely tested for forage quality (e.g. fiber, protein and calculated digestibility values) to determine their nutritional value for feeding purposes. If those same cored samples used for forage quality analysis could also be used for nutrient management purposes, it would greatly simplify the process of tissue testing and encourage more careful nutrient management. Using this method, growers may be able to ‘pick up’ nutrient deficiencies that would otherwise go undetected.

In this project we intend to: 1) Evaluate the feasibility of using whole plant samples (simulated cored-bale hay samples) to determine the nutrient status of alfalfa fields to guide fertilization practices; 2) compare 3 different plant tissue sampling methods for nutrient monitoring; 3) quantify the phosphorus, potassium, sulfur, boron and molybdenum tissue concentration in alfalfa plant tissue over time as the crop matures from early bud growth stage to 10% bloom and correlate these values with stage of growth and crop height; 4) determine alfalfa yield response from phosphorus, potassium and sulfur fertilization; 5) develop critical plant tissue concentration values for alfalfa samples at different maturities; and 6) evaluate the accuracy of NIRS analysis to determine nitrogen, phosphorus, potassium, sulfur, boron and molybdenum concentrations.

Twelve commercial alfalfa fields will be sampled each of two years in the Intermountain area, Sacramento/San Joaquin Valley and the High Desert. Three different sampling methods will be evaluated as to their ability to reflect the nutritional status of fields. Sampling methods include fractionated plants (current UC method), top 6 inches (used in other states), and whole plants (simulated cored-hay samples method). Cored bales samples as well as samples obtained from previous research will also be analyzed using NIRS to evaluate the feasibility of using this technology for mineral analysis. Fertilizer rate studies will be established to determine crop response to applied phosphorus, potassium and sulfur. Plots will be sampled from early bud growth stage to 10 percent bloom to quantify changes in the nutrient concentration in plant tissue with advancing maturity. This will allow us to develop critical tissue concentrations for different sampling methods for a range of alfalfa maturities.

The overall goal is to develop an alfalfa tissue testing protocol that is simple to use and sufficiently accurate so that nutrient analysis can become a routine component of forage quality testing. Those who would benefit include alfalfa growers, consultants (PCA’s, CCA’s and Farm Advisors), fertilizer companies, and testing laboratories. Dairies and consulting firms could use this information to assist with dairy nutrient management plans and monitoring protocols. Public agencies concerned with nutrient management on cropland and dairies would be able to use this information to promote better nutrient stewardship and monitoring.
C. JUSTIFICATION

Problem
Alfalfa is the largest acreage crop in California, at approximately one million acres and is grown in nearly every county in California from the Mexican border to the Oregon border. Proper nutrient analysis for alfalfa is important for several reasons. First, timely and cost-effective decisions are needed to make sure that yields are not reduced due to deficiencies—these yield losses have major economic consequences for farmers. Secondly, farmers should assure that fertilizers are not over-applied; to carefully match crop need with fertilizer practices, since over-application of fertilizers often result in environmental impacts. Thirdly, there is increased need to monitor nutrient levels in plants for the purposes of managing dairy (and other) waste material applied to forage crops. Alfalfa is increasingly used for recycling and utilizing dairy wastes, and better management would enable monitoring (for regulatory purposes or otherwise) to be accomplished.

Plant tissue testing is the most accurate means to assess the fertilization needs of alfalfa. However, at the present time, most alfalfa growers do not conduct tissue testing, in spite of long-standing UC recommendations. We estimate that less than one percent of the alfalfa fields are tissue tested in any given year. The current recommendation is to sample the standing crop at 10% bloom and fractionate the sample into three parts (tops, mid-stem, and mid-stem leaves). Stems from the middle third of the plant are analyzed for phosphorus and potassium, the leaves of the middle third portion are analyzed for sulfur and the top one third of the plant is analyzed for boron and molybdenum. Unfortunately, this process may be too cumbersome and confusing for routine analysis. It is also expensive, as many labs charge producers for three separate samples. Plant tissue testing is far more accurate than soil analysis because it better reflects nutrient uptake and avoids limitations of soil sampling and nutrient extraction. In spite of the superiority of plant tissue testing, it has not been widely adopted. Improved methodologies which encourage the use of tissue testing will result in better fertilization practices.

To develop better, more practical sampling methods it is necessary to have methods that are 1) rapid and convenient, 2) standardized and interpretable with tables to result in fertilizer recommendations, and 3) standardized with regards to plant sampling protocols, particularly stage of growth. Our goal is to develop a sampling protocol for alfalfa that accomplishes these objectives.

Many alfalfa growers routinely take cored samples of haystacks to assess the forage quality (ADF, NDF, CP and DM) of their alfalfa. Forage quality analysis is typically required to market alfalfa to the dairy industry. There are 17 certified hay testing laboratories in California (certified by NFTA), the most of any state, and tens of thousands of samples are routinely analyzed each year for quality at these labs. To encourage adoption of tissue testing, we hope to further evaluate the feasibility of using a cored-hay sample for both nutrient and forage quality analysis. This could be incorporated into routine testing practices and greatly simplify the tissue analysis process and reduce costs for growers. Most importantly, a streamlined method such as this would enable growers to easily adapt tissue testing for P management (and other nutrients). Also, due to the fact that core sampling of hay stacks represents a wide range of plant material (greater than standing crop samples), it may be more successful at representing the average nutrient concentration of a field than other sampling methods.

Forage quality analysis of cored-bale samples can be done using “wet chemistry” or near infrared reflectance spectroscopy (NIRS) analytical techniques. Many commercial laboratories use NIRS for their forage quality testing. NIRS is an accurate, precise, and rapid alternative to wet chemistry procedures for determining the concentration of chemical compounds such as ADF and NDF in alfalfa forage. The method utilizes reflectance signals resulting from molecular
bonds between carbon, nitrogen, hydrogen, and oxygen. Calibration is required to correlate the spectral response of each sample at individual wavelengths to known chemical concentrations from laboratory analyses. Wet chemistry is traditionally used for nutrient analysis but many laboratories are currently reporting nutrient levels using NIRS as well. While this is a routine practice of some laboratories, the accuracy of NIRS to determine the concentration on mineral nutrients is not known and many feel may be suspect. NIRS is typically used to analyze carbon containing compounds rather than minerals. The speed, ease-of-use and repeatability of NIRS technology could greatly encourage the adoption of tissue testing of alfalfa, but research is clearly needed to assess the accuracy of NIRS for nutrient analysis before its use could be promoted.

The cored-bale sampling technique shows significant advantages for routine analysis to detect nutrient deficiencies and guide fertilization practices. In addition to helping guide fertilization practices, cored bale sampling for nutrient content will be extremely useful for dairies that are required by the Regional Water Quality Control Board to develop Nutrient Management Plans. These plans require routine analysis of N, P and K for nutrient management purposes. It is our objective to harmonize these techniques so that they could meet both fertilizer management and nutrient management goals.

CDFA/FREP Goals
This project compliments the CDFA/FREP goals by developing an improved diagnostic tool for predicting crop nutrient response (plant tissue testing using cored bale samples) that can aid in making fertilizer recommendations. This will result in wiser fertilizer management and improved profitability for growers. A more accurate method for assessing fertilizer needs would go a long way toward improving profitability for such a large acreage crop that largely does not currently utilize diagnostic tools.

This project also compliments the CDFA/FREP goals by updating nutrient requirements to improve crop yield. Fertilizer rate trials will be conducted to correlate different fertilizer amounts and plant tissue levels with alfalfa yield. Critical nutrient levels and fertilizer application quantities to achieve those yields will be determined.

Impact and Long-Term Solutions
This project has the potential to significantly improve nutrient management in alfalfa throughout California and beyond to other alfalfa-producing states. Using cored bale samples as a tool to predict fertilizer requirements will enable growers to include these measurements routinely as a component of their regular forage quality testing program. This will encourage wider adoption of tissue testing to guide fertilization practices. Currently the biggest problem with tissue testing is that it is one more extra activity that often is overlooked by the grower—but that problem may be solved if core samples from bales could be analyzed for nutrient analysis as a part of routine testing by labs who measure forage quality. If NIRS proves sufficiently accurate to determine nutrient concentrations in alfalfa, it will further increase adoption and speed up the analytical process. Baler sampling of alfalfa will also be extremely useful for dairies that are required by the Regional Water Quality Control Board to develop Nutrient Management Plans. These plans require routine analysis of P, K and N for nutrient management purposes. It is our objective to harmonize these techniques so that they could meet both fertilizer management and nutrient management goals.
Related Research and/or Education Efforts

Schmierer et al. (2005) and Orloff et al. (2008) reported that critical values for alfalfa tissue samples were highly influenced by alfalfa growth stage at the time of sampling. Concentrations of P decline rapidly as the crop grows and matures. Therefore, the critical value (the level at which fertilization is required), should be much higher for bud-stage alfalfa compared with 10% bloom alfalfa. Current UC fertilization recommendations are based on sampling at 10% bloom (much later than most grower harvest to produce dairy-quality alfalfa) and do not adequately take into account the effect of crop maturity on interpretation of the plant tissue testing values. The effect of maturity on the concentration of other nutrients in alfalfa, such as potassium and sulfur, is not known. Therefore, methods are needed to standardize sampling protocols, so that the influence of plant maturity can be factored into the interpretation of plant tissue concentrations. This project should assist in the standardization of the in-field sampling techniques.

Initial research was conducted by Orloff et al. (2008) to evaluate the reliability of cored-bale samples to guide fertilization practices. Results to date indicate that there is significant potential for this approach to tissue sampling. Cored-bale samples correlated well with the standard UC tissue-testing protocol using standing plant grab samples fractionated into different plant parts. This research was conducted in the Intermountain area and additional research is needed to validate these findings and to test the concept in different areas of the state. Initial results were presented at county grower production meetings, the California Alfalfa Symposium, the Washington State Hay Growers’ Conference, the National Alfalfa Symposium, and combined conferences of FREP and the Western Plant Health Association in Sacramento and Modesto. The results have been well received by alfalfa producers and industry.

Contribution to Knowledge Base

This project would create a valuable new set of data that is largely not available currently. We should be able to compare several common methods for sampling the major nutrients in alfalfa, assess the impact of plant growth stage on nutrient concentration, and evaluate a new proposed method of tissue testing which could enable expanded use of tissue testing of alfalfa for fertilizer management. The results could help standardize plant tissue testing protocol beyond California. This project will show the relationship between wet chemistry for nutrient analysis and NIRS. This information will be useful not only for alfalfa growers but perhaps for other commodities as well. Knowing the range in nutrient composition of alfalfa at different times of the year for different growth stages will assist with Nutrient Management Plans and having such a data base may help negate the need to sample at every cutting.

Grower Use

The primary audience will be alfalfa hay producers and we will work closely with Pest Control Advisors, Certified Crop Advisors, forage testing laboratories and fertilizer sales personnel. This project may open up significant opportunities for improved crop monitoring by growers, fertilizer companies, CCAs and PCAs, as well as opportunities for more sample analyses by labs. Dairy producers may be a secondary beneficiary of this project because the results could help them more easily comply with Water Resources Control Board requirements.
D. OBJECTIVES

In this project, we intend to:

1) Evaluate the feasibility of using whole plant samples (simulated cored-bale hay samples) to determine the nutrient status of alfalfa fields and to guide fertilization practices;

2) Compare 3 different plant tissue sampling methods for nutrient monitoring (top 6 inches, fractionated plant, and whole plant sample) as to their ability to reflect the nutritional status of fields;

3) Quantify the phosphorus, potassium, sulfur, boron and molybdenum tissue concentration in alfalfa plant tissue over time as the crop matures from early bud growth stage to 10% bloom and correlate these values with stage of growth and crop height;

4) Determine alfalfa yield response from phosphorus, potassium and sulfur fertilization;

5) Develop critical plant tissue concentration values for alfalfa samples taken from baled hay;

6) Evaluate the accuracy of NIRS analysis to determine nitrogen, phosphorus, potassium, sulfur, boron and molybdenum concentrations.

7) The results of this research and the resulting recommendations will be disseminated to alfalfa producers, consultants and allied industry through field days, regional alfalfa production seminars, Alfalfa Symposia in California and other states, newsletter articles, trade magazines, and through the UC Alfalfa Workgroup website.

E. WORKPLANS AND METHODS

Experimental Plans.

To meet these objectives the following Tasks will be completed.

1. Sampling Commercial Alfalfa Fields to Compare Tissue Testing Protocol with Wet Chemistry Methods and NIRS at different Growth Stages

Approximately 12 commercial alfalfa fields will be sampled each year in three different alfalfa production regions (Intermountain area, Sacramento/San Joaquin Valley and the High Desert). Fields will be selected that are believed to represent a range of nutrient levels. Samples will be collected at all three cuttings in the Intermountain area and spaced throughout the season in the Central Valley and High Desert to quantify the effect of the season (or cutting) on nutrient concentration. Plant tissue samples will be analyzed for nitrogen, phosphorus, potassium, sulfur, boron and molybdenum (the nutrient deficiencies known to occur in alfalfa in California). Three types of plant tissue samples will be collected. 1) Fractionated plant sample according to the standard UC protocol (as described in Irrigated Alfalfa Management) with three different plant parts. The stems from the mid-third portion will be analyzed for PO4-P and K. The leaf portion of the middle third will be analyzed for SO4-S, and the top third portion for boron and molybdenum. 2) The top 6 inches of the alfalfa plant (method used in other alfalfa-producing states) will be analyzed for N, total P, K, total S, boron and molybdenum. 3) Whole plant samples (used in some states and comparable to bale samples) will be analyzed for the same nutrients as the top 6-inch samples. Each of these samples will be taken at each of three
maturities: 1) Early Bud, Late Bud, and Early (10%) Bloom (this is the current standard for all states).

Soil samples will also be collected from each of the fields. The soils will be analyzed for standard nutrients. This task will allow us to determine the relationship between the different sampling methods and compare the results with soil analyses.

The soil samples will be analyzed by the UC DANR Analytical Lab. Soil samples will be analyzed for nitrate N, ammonium N, pH, P, K, S, B, Mo and Mg. All the plant tissue analyses mentioned above will be done using wet chemistry methods by the DANR Analytical Lab. The whole plant samples (#3 above) will be analyzed using NIRS for N, P, K, S, B and Mo. The NIRS analysis will be done by Dan Putnam’s laboratory at UC Davis and at a commercial laboratory experienced with NIRS (JL Analytical Services, Inc).

2. NIRS Analysis of Existing Samples.
Previous research (mentioned above in related research) was conducted in the Intermountain area using similar protocol to that mentioned above in Experimental Plan #1. A total of 117 samples were collected over 2 years from 39 fields ranging in nutrient status from extremely deficient to very high. The samples were analyzed using wet chemistry techniques by the DANR Analytical Lab. We have retained the samples from that research. Analyzing those samples for total P, K, total S, B and Mo will enable us to assess the value of NIRS for estimating the mineral content of forages and to help establish a calibration that can be used to analyze other samples. NIRS analysis will be performed by Dan Putnam’s laboratory and by JL Analytical Services, Inc.

3. Laboratory Samples for NIRS and Wet Chemistry Validation and Calibration
We will request 100 samples from several laboratories throughout California that perform forage quality analysis of alfalfa hay. The samples selected will be from various locations from throughout the alfalfa production-regions of the state and will represent fields with a wide range of nutritional status. The samples will be analyzed for the same nutrients as in #2 above. This will allow us to validate the relationship between NIRS and wet chemistry that we develop from #1 and 2 above and to further refine the calibration.

4. Fertilizer Rate Studies
Fertilizer response trials will be conducted to establish different plant tissue levels and correlate yield with nutrient concentration in plant tissue. Each trial will have 5 different rates (unfertilized and 4 rates) with four replications. Potassium and sulfur rate studies will be conducted in the intermountain area (phosphorus rate studies have already been completed in the Intermountain area). Phosphorus and potassium rate studies will be conducted in the Central Valley and a phosphorus rate study in the High Desert. We will take 3 sequential standing plant samples starting in early bud stage until harvest at approximately 10 percent bloom. Three sampling methods will be used: fractionated samples (UC protocol), top 6 inches (method popular in other states) and whole plant samples (similar to cored bale samples). Growth stage and plant height will be recorded at each sampling time. Intermountain trials will be sampled for the first and second cutting. Three cuttings from throughout the season will be sampled in the Central Valley and High Desert. Yield data will be collected to determine the yield response to applied P, K, and S and to correlate those yield levels with plant nutrient concentration. These trials will document the change in nutrient concentration with advancing alfalfa maturity for the three different sampling methods. This research will provide information needed to develop critical tissue levels at different alfalfa maturities for different sampling techniques.
Analysis. Regression analysis will be used to compare sampling methods, determine the relationship between them and to quantify the degree of variation with each sampling method. Analysis of sources of variation, including within-field variation, will enable us to determine whether one method is less variable (and therefore more predictive) than another measurement. The NIRS data collected in Experimental Plans 1 and 2 above will allow us to assess the relationship between NIRS estimation of nutrient concentration and wet chemistry. If NIRS shows promise for nutrient analysis, the data collected will allow us to strengthen the calibration. Further validation and calibration will occur in Experimental Plan 3. Data collected from the fertilizer rate studies will be subjected to Analysis of Variance Analysis (ANOVA) to determine the effect of fertilizer rate on forage yield. Regression analysis will be used to assess the effect of plant maturity on nutrient concentration levels in plant tissue. We will compare the plant tissue levels and the alfalfa yield associated with that level to determine whether existing critical values need adjustment. Tracking nutrient levels over time with advancing alfalfa maturity will allow standardization to account for the effect of plant maturity and will enable a correct interpretation of the nutrient status of a crop.

A complete detailed summary of the projected sampling regime is provided in the Budget section. This study is projected as a 2-year study.

Tasks:
A meeting will be held upon receipt of the grant to develop final protocols for sample numbering, discussion of field identification, and sampling scheme. Datasheets and sample collection materials, purchase of sampling equipment, and identification of laboratory methods and grower cooperators will be completed.

Task 2. Fertilizer Rate Studies and Data Collection. (2011, 2012)
Subtask a. Fertilize plots in late winter to establish the plots.
Subtask b. Collect plant tissue analysis starting in late spring at early bud stage up to harvest.
Subtask c. Harvest 2–3 cuttings per year depending on location.

Task 3. NIRS Analysis of Previous Samples. (Winter–Spring 2011, 2012)
Nutrient composition (N, P, K, S, B, Mo) of samples collected in previous studies in the Intermountain Region will be analyzed using NIRS at the Putnam Laboratory and at JL Analytical.

Subtask a. Intermountain (8–10 Sample Sets)
Subtask b. Central Valley (8–10 Sample Sets)
Subtask c. High Desert (8–10 Sample Sets)
Samples will be collected according to the sampling schemes beginning April of 2010 (depending upon location). These will be carefully identified, processed and stored for later analysis.

Task 5. Laboratory Analysis. (late summer/fall, 2011, 2012)
Subtask a. Grinding and processing of samples
Subtask b. Nutrient analysis (N, P, K, S, B, Mo) of each sample according to widely accepted protocols for each parameter
Subtask c. Forage Quality analysis (ADF, NDF, CP), according to the protocols developed in Task 1.

  Subtask a. Data entry, quality control of raw data
  Subtask b. Statistical analysis, regression analysis, correlations
  Subtask c. Graphing of data, finalization of tables and graphs expressing the data
  Subtask d. Interpretation, analysis of graphs, tables, development of recommended critical levels at different plant maturities.

Final Report June 2013. As appropriate, we will feature these trials at UC-sponsored field days (in counties and at the UC Davis Alfalfa Field Day), and present data at county-based, and state-wide and regional meetings such as the California Alfalfa Symposium and the Western Alfalfa Conference. As the project progresses, we will develop newsletter articles, updates and reports to keep the public informed as to the results of the project. Articles and the accompanying recommendations regarding sampling protocol and interpretation of values will be posted on the UC Alfalfa Workgroup webpage.

F. PROJECT MANAGEMENT, EVALUATION AND OUTREACH

Management. Project management (sample management, development of protocols, etc.) will be accomplished by the 2 PIs in cooperation with growers, the California Alfalfa & Forage Association, CCAs and Farm Advisors. PI’s will work closely with the Farm Advisor cooperator, Andre Biscaro, and Mike Wolf from JL Analytical, LLC to establish the protocol and coordinate activities. Task 1 includes a meeting to finalize these protocols.

Evaluation. The milestones for the project will include records for the Tasks outlined under E above. This type of project involves a sequential process of planning, sample collection, sample analysis, data management and analysis, interpretation and outreach, each of which has its own milestone. At the completion of the first year in November, we will have a considerable amount of data (but not completed) based upon the samples collected earlier in the year. We have an initial assessment of the accuracy and usefulness of the different sampling methods. At that point we will also know if NIRS analysis looks promising for nutrient analysis. Protocol could be altered slightly after analysis of the first year results if deemed necessary. We hope to impact the way in which fertilizer needs are determined for alfalfa by promoting plant tissue testing as a routine practice along with the already standard practice of forage quality testing for marketing purposes. Evaluation of these research data will be based upon the degree to which new techniques are adapted by alfalfa growers, consultants and laboratories. Upon completion of the project, we could survey clientele and California laboratories to determine whether the recommendations developed through this research are useful in the development of their nutrient management programs and if there is an increase in tissue testing of alfalfa.

Outreach. A primary outlet of this research data is the California Alfalfa Symposium (which is periodically combined with the Western Alfalfa & Forage Symposium) held each December. This is important, since it is considered the most important annual gathering of the alfalfa industry in California, and is attended by between 350 and 700 people annually. Additionally, the Proceedings of this symposium are a premier source of information about irrigated alfalfa, and available on the web at: http://alfalfa.ucdavis.edu/+symposium/archive.html. In addition to
these venues, we plan to present the research findings at field days and local winter meetings. The UC Davis Alfalfa Field Day is held each year in May, and county-based or regional alfalfa meetings are typically held in the fall or in the winter at different locations throughout California. Near the completion of the study, we hope to write a journal article, along with popular articles which will describe the outcome of the study. A presentation will be made at the FREP conference, held each year in the fall (depending upon the organizers).
**Budget Explanation:** This is an extension of the project with no change in budget. Funding is requested primarily for the extensive plant sampling along with soil sampling required to meet the objectives of the project. Support is requested for summer labor assistance for Farm Advisors (co-PI in Intermountain area and cooperator in the High Desert) and a Staff Research Associate II for the research sampling and harvesting in the Central Valley and for laboratory work with the NIRS. The SRA II is Chris DeBen and the summer field technicians are to be hired. The only supplies/non-inventory equipment we need is samplers for doing the sampling in a scientific manner, and miscellaneous supplies (sample bags, flags, etc.). Lab analysis details are provided below. While no other matching funding is listed, researchers have contributed three years of data collection to this project, before funding was secured. Some travel is needed for collection of samples, and for attendance at the FREP conference and other venues where the data will be presented (outreach) for the PIs and cooperators.