Non-Technical Summary

Alfalfa as a livestock feed has direct value in rations for a diversity of livestock. Alfalfa provides multiple ecosystem services, and is an important rotation crop for breaking insect pest, weed, and disease cycles. Alfalfa reduces soil erosion, captures nutrients from annual crop fields that can contaminate surface and ground water, and promotes soil carbon sequestration. The value of the N from alfalfa biological N fixation to annual crops represents a fertilizer saving of more than $80/acre. We now have a unique opportunity to enhance alfalfa use in livestock rations and to increase its presence on the agricultural landscape. The development of a new reduced-lignin alfalfa has potential to provide a higher digestibility feed that will increase its feeding value and profitability of its use in rations. A transgenic, reduced-lignin alfalfa, named HarvXtra and marketed by Forage Genetics International, contains about 12-18% less whole plant lignin than standard varieties. The new transgenic reduced-lignin alfalfa was produced by deactivating enzymes in the lignin synthesis pathway. Dairy cow feeding trials with this alfalfa forage in the ration showed increased milk production of 1.2 kg/cow/day compared to forage from conventional alfalfa. Lignin concentration is highly correlated with forage digestibility, such that relatively small changes in lignin concentration will significantly influence fiber digestibility. The greater the reduction in lignin, the greater the impact on fiber digestibility, therefore transgenic reduced-lignin alfalfa is expected to have the greatest impact on animal performance. This new technology needs to be evaluated under diverse harvest regimes designed to produce forage with yield and quality to meet producer goals, including systems with alfalfa in mixture with perennial grasses. Use of new reduced-lignin alfalfa varieties has potential to be very advantageous to growers. At any given maturity stage, reduced-lignin alfalfa will have greater cell wall digestibility and greater feeding value than conventional varieties. In addition, the new technology also provides growers management flexibility to delay harvest to a later stage of maturity. It provides a wider harvest window without loss of digestibility. Increasing diversity in cropping systems helps to balance profitability and environmental concerns. We propose to increase cropping systems diversity by diversification in time and with diversity in space. Throughout the northern and transition zone regions, alfalfa is frequently grown in binary mixture, and new grasses provide unique opportunities to increase use of alfalfa.
methods. For example, a survey of alfalfa acreage in New York State in 2014, determined that over 84% of the alfalfa acreage in NY is planted with a perennial grass, compared to many regions of the Midwest where alfalfa is primary grown in monoculture. Advantages of alfalfa-grass mixtures compared with alfalfa monocultures include:

1) Reduced chance of alfalfa heaving and winter kill.
2) Less concern over application of animal manures.
3) Improved soil and water conservation.
4) Maintain a full stand of forage for more years as the alfalfa component declines.
5) Increased resistance to alfalfa insect pests.
6) Faster drying of mixed alfalfa-grass forage in the field.
7) Reduced bloat potential of grazed alfalfa.
8) Reduced soil structure damage by grazing animals.
9) Provide more balanced nutrition to livestock than a pure crop of either alfalfa or perennial grass.

While regional seeding rate recommendations exist for establishment of desired proportions of alfalfa and grass in mixtures, often the outcome of mixed seedings is unpredictable. In addition, the proportion of alfalfa and grass in mixtures is dynamic and changes from season to season and year to year. However, estimating the alfalfa proportion of mixtures is essential for producers for harvesting at optimum forage quality and for researchers for evaluating results. While many producers recognize the importance of grass-alfalfa mixtures, determination of the proportion of grasses and alfalfa is challenging. Most mixed stands are variable, so visual estimation of composition or making collection of a representative sample for manual separation are both difficult.

Knowing the alfalfa-grass proportion in a mixture is a critical factor influencing use of mixed stands because this proportion strongly affects neutral detergent fiber (NDF) concentration. Neutral detergent fiber is critical in formulating diets for lactating dairy cattle, and within a species, the NDF content is well related to the digestible NDF content. These are the primary reasons that we focus on evaluation of NDF as a basis for harvest. Maximum alfalfa height by itself has been shown to be an accurate method of estimating neutral detergent fiber (NDF) of pure alfalfa, and also NDF of alfalfa-grass mixtures, as long as alfalfa proportion is known. The NDF equation for alfalfa-grass mixtures is the cornerstone of the Alfalfa-Grass Evaluation System (AGES) for assessing alfalfa proportion and alfalfa quality by producers, which will be assessed by this proposed project. These equations are currently being evaluated at McGill University, Montreal, QC, to test their robustness. Optimum NDF concentrations for alfalfa and pure grass species have been established, and we can estimate optimum NDFD content based on NDF for spring growth.

Forage properties, such as species composition and NDF content, can be evaluated nondestructively in alfalfa-grass stands. We have determined that it is possible to accurately estimate alfalfa proportion of alfalfa-grass mixtures in the field, using digital image analysis. Multiple cameras, including Android and iPhones cameras, were used to collect images. Local binary patterns (LBP) applied to pixel tiles is proving to be successful.

Preliminary model testing demonstrated potential for accurate results under a range of conditions. This photo assessment process is somewhat similar to that involved with facial recognition software. Photos taken with a cell phone will be able to accurately estimate alfalfa proportion. Using a smart phone or computer, the alfalfa proportion information can be combined with alfalfa maximum height information to immediately generate the current mixed stand NDF content. This information can be used to estimate the number of days it will take to reach optimum forage quality, because we know the average rate of decline in NDF and NDFD per day. Model development is now complete, with additional sampling, testing, and validation is occurring in 2015. Our planned work on evaluation of alfalfa-grass mixtures in diverse environments provides a unique opportunity to evaluate this new technology in diverse environments, and over multiple alfalfa-grass combinations.

Use of new reduced-lignin high digestibility alfalfas is a foundational approach to increasing profitability, value, and use of alfalfa grown in pure stands (monoculture) and mixture with improved quality perennial grasses. Validation of a method to determine alfalfa proportion in mixed stands will allow both researchers and alfalfa producers to accurately evaluate this crucial parameter for mixtures. Direct beneficiaries of this research will include livestock farmers feeding alfalfa and alfalfa-grass mixtures, hay producers, and hay marketers. Greater acreage of alfalfa in monoculture and mixtures will benefit all citizens from improved water quality and healthier agroecosystems.

Accomplishments

Major goals of the project

Project Objectives

Objective 1: We will evaluate forage yield and forage quality of reduced-lignin alfalfa in monoculture and in binary stands when subject to two harvest regimes focused on forage yield or quality.

Experimental design: Randomized complete block with treatments in a split-split plot arrangements with four replicates.

Whole plot

Methods: A two year (seeding plus first production year) experiment is proposed from 2015-2017 at Cornell University
Caldwell Field research farm, Univ. of Minnesota Research and Outreach Center, Rosemount, and Univ. of Kentucky Spindletop research farm. A third year harvest year is planned, but not included for funding in this proposal. Plots will be established in April or early May of 2016 by broadcasting inoculated seed and cultipacking or seeding with a plot drill into a prepared seedbed. Alfalfa monocultures will be seeded at 16.8 kg pure live seed/ha and alfalfa in mixture will be seeded at 11.2 kg/ha. Grasses will be seeded at approximately 6 kg/ha, with all three grasses seeded at the same number of pure live seeds/ha. Subplot size will be 1.8 x 6.1 m. Weeds in monoculture will be controlled using a post-emergent herbicide applied according to herbicide label. We will scout for insects known to be detrimental to alfalfa yield and quality and apply appropriate insecticides.

Alfalfa harvest managements will be applied beginning in the seeding year when alfalfa reaches the designated stages of maturity but will conclude by early September at all locations. They will be applied again beginning in the spring in the year following seed year. No fall cutting will occur either year.

Alfalfa proportion in binary mixtures will be determined using techniques described under Objective 2. Forage yield will be measured at each harvest in the seeding year and year following seeding by harvesting a 0.9 by 5.5 m area in each plot to a 0.5 cm height. A 1 kg subsample of standing forage will be collected, dried, and yield expressed on a dry matter basis. Forage quality will be measured by manually taking a sample from 0.2 m² from standing forage at time of plot harvest and drying at 60°C for 3 days. Samples will be taken in the seeding and year following seeding. Binary mixtures will be separated in alfalfa and grass components, and analyzed separately. Analysis will be assessed on the subsample by determination of crude protein, acid detergent fiber, NDF, NDFD, and mineral concentration (P, K, Ca, Mg) using a combination of near infrared reflectance spectroscopy (NIRS)(Sheaffer et al., 1998) and standard laboratory chemistry for calibration. Using forage quality variables, we will calculate the RFQ (Relative Forage Quality) forage quality index.

Alfalfa maturity at harvest will be quantified at each harvest using the mean stage by weight method (Kalu and Fick, 1983). Grass maturity at harvest will be quantified using a simplified staging system (Parsons et al., 2006a). Stand evaluation will be determined by counting plants in 0.2 m² areas in the 4 weeks following seeding and in the fall of the seeding year and in the spring and fall in the year following seeding. Botanical composition of the monocultures and binary mixtures will be measured at each harvest by methods described in Objective 2. Plant foliar disease will be determined by visually rating percent defoliation of 10 randomly harvested stems will be measured from each plot immediately prior to each forage harvest. The primary foliar diseases will be identified by symptoms and/or pathogen isolation.

**Statistical and economic analysis:** Analysis of variance using least squares will be used to determine significant differences in treatments. Treatments will be tested as fixed effects while blocks nested within locations will be tested as random effects. Statistical analysis will be done using program SAS or R.

We will conduct a spreadsheet-model cost-benefit analysis for each treatment combination. We will determine the returns of each treatment using yield and forage quality data, and then subtract production costs and added costs to identify the system that maximizes profits. We will calculate the returns using variable return and input cost scenarios.

**Timeline:** We are asking for funding for two years, from 2015 to 2017, however, we plan on harvesting this study in 2018. Field activities will be conducted in 2016 and 2017. Fall 2015: plots will be prepared and fertilized at all locations. Winter 2015: Final plot plans developed; seeds of all alfalfa and grass varieties will be obtained. Spring 2016: Planting of seeds. Summer and Fall 2016: Cutting treatments will be applied. Forage yield will be determined and forage quality samples collected. Plant populations will be determined. Fall and Winter 2016-2016: Analysis for forage quality and statistical analysis of seeding year data. Spring, Summer, 2017: Intensive harvest schedule applied over all plots, forage yield measured and forage quality samples collected, plant populations measured. Fall and Winter 2016-2017: Laboratory analysis for forage quality and combined 2-year statistical analysis of data.

**Objective 2:** Validate a system (AGES: Alfalfa-Grass Evaluation System) for assessing alfalfa proportion of alfalfa-grass mixtures in Objective one, and also for assessing alfalfa proportion and alfalfa quality by producers, for optimum forage quality and effective management of stands.

Samples (0.8 m diameter circle) of binary alfalfa-grass mixtures will be collected across NY State in 2016, after taking photos, and recording stand height information, and then separated into species components for analysis. A minimum of 100 samples will be collected for validation purposes. A range of grass species will be included in these samples. These samples will allow both the validation of the alfalfa proportion from photos software, and will allow further testing of our NDF prediction equations.

**Objective 3:** Develop an extension education program to help educate all stakeholders about the production and management requirements for reduced-lignin alfalfa and alfalfa-grass mixtures and the quality and feeding benefits of this new trait, and also educate stakeholders about AGES.

The Education and Outreach portion of this project is a cooperative partnership between state forage crop extension specialists in Minnesota, Kentucky, and New York. Together, these organizations will provide a comprehensive education and outreach effort that will be available to a diverse group of stakeholders: (i) farmers and crop advisors, (ii) extension educators and state agency personnel, (iii) the scientific community, and (iv) the general public.

**What was accomplished under these goals?**
The development of reduced-lignin alfalfa gives us a unique opportunity to enhance alfalfa use in livestock rations and to increase its presence on the agricultural landscape. Higher quality alfalfa means increased feeding value and profitability. This new technology needs to be evaluated under diverse harvest regimes designed to produce forage with yield and quality to meet producer goals, including systems with alfalfa in mixture with perennial grasses. This information will primarily benefit all alfalfa producers.

Objective 1. Yield and quality of reduced-lignin alfalfa in monoculture and in binary stands with perennial grasses. All higher or lower data comparisons mentioned below are significant (p < 0.05).

Seeding year harvests, 2016. Plots in MN were not sufficiently established for any seeding year harvests for yield and quality. WL355RR alfalfa yielded 9% higher than HarvXtra in KY and 15% higher in NY. Alfalfa-grass mixtures yielded 36% higher than pure alfalfa in KY, with no differences in NY. Quality parameters were weighted by yield over the season, and bud and flower harvest stages were averaged. HarvXtra was 7% higher in fiber digestibility (NDFD) than WL355RR in KY and 6% higher in NY. HarvXtra was 8% lower in lignin than WL355RR in KY and 15% lower in lignin in NY. End of season grass in mixtures was orchardgrass (OG) 19%, meadow fescue (MF) 4%, and festulolium (FES) 23% in KY, while OG was 19%, MF 9%, and FES 2% of mixtures in KY. Orchardgrass was 15% higher in NDFD than FES, and 28% higher than MF in KY. Conversely, MF was 8% higher in NDFD than OG, and 3% higher than FES in NY. To demonstrate regional differences, at the flower stage of growth for alfalfa, HarvXtra was 57% NDFD in NY and 46% NDFD in KY, while MF was 77% NDFD in NY and 40% NDFD in KY.

Harvests in 2017, 2016 spring-seeded studies. MN harvested only at bud stage with 3 harvests. NY harvested at bud and flower stages with 4 harvests each. KY harvested at bud and flower stages with 5 harvests for bud stage and 4 harvests for flower stage. WL355RR was at a higher morphological mean stage than HarvXtra in all three states, leading to higher alfalfa fiber content (NDF, ADF) in all three states. Alfalfa-grass mixtures were 7% higher yielding in NY, but not different from pure alfalfa in KY and MN. Over harvest schemes, WL355RR yielded 13% higher in MN and 19% higher in KY than HarvXtra, with similar yields of the two alfalfas in NY. HarvXtra was 4.1% higher in NDFD than WL355RR in KY, 5.5% higher in NY, and 8.2% higher in MN. HarvXtra was 15% lower in lignin than WL355RR in KY, 15% lower in NY, and 22% lower in MN. Weighted average grass percentage in mixtures over the season varied by region: 31% MF, 61% OG, and 31% FES in NY, 10% MF, 26% OG, 15% FES in KY, and 46% MF, 30% OG, and 18% FES in MN. Grass quality also varied by region. In KY, FES was 8% higher in NDFD than MF and 5% higher than OG. In NY, MF was 11% higher in NDFD than OG and 10% higher than FES. In MN, grasses did not differ in NDFD or lignin. FES was 16% lower in lignin than MF in KY, and 15% lower than OG. Conversely in NY, MF was 22% lower in lignin than OG, and 10% lower in lignin than FES. Harvests in 2017, 2016 late summer seeded studies. HarvXtra, Hi-Gest 360, and WL356HQ.RR were seeded in monoculture or with FES, OG, TetraMF, or BARFpF32 MF. Four harvests were taken in NY at a bud stage, and three harvests were taken in MN at a bud stage. Yield of mixed stands was similar to alfalfa monocultures at both sites. In NY, HarvXtra was 8% higher yielding than Hi-Gest, while both Hi-Gest and WL were 12% higher yielding than HarvXtra in MN. HarvXtra was lower in fiber (NDF, ADF) than other alfalfas at both sites, agreeing with results from the spring-seeded studies. HarvXtra was 4.0% higher in NDFD than Hi-Gest, and 5.3% higher than WL in MN. HarvXtra was 8.1% higher in NDFD than Hi-Gest, and 7.7% higher than WL in NY. HarvXtra was 11% lower in lignin than Hi-Gest, and 13% lower in lignin that WL in NY, and 16% lower than Hi-Gest and 18% lower than WL in MN. HarvXtra mixed stands had 24% more grass than other alfalfas in NY, but all alfalfas were the same for grass% in MN. In NY, MF mixed stands averaged 44% grass, while OG was 41% and FES 32%. In MN, MF cultivars differed significantly, with 43% grass for BARFpF32 and 14% for TetraX. Orchardgrass was 7% and FES 9% grass in mixed stands. Grass NDFD differed among all grasses in NY: Tetrax 81%, BARFpF32 79%, OG 74%, and FES 73%. Both MF cultivars were 27% lower in lignin than OG, and 12% lower than FES. Conclusions. Alfalfa cultivar quality differences were relatively consistent over regions, while grass species quality varied greatly by region. HarvXtra was consistently much lower in lignin and somewhat higher in NDFD than other alfalfa cultivars evaluated, although HarvXtra tended to yield somewhat lower than other cultivars. Meadow fescue was consistently higher in NDFD than other grasses in NY, but that was not true for other regions. Regional evaluation of grass species and cultivars is required.

Objective 2. Validate an alfalfa-grass evaluation system. Photos were taken of alfalfa-grass stands in 2015, 2016 and 2017 to help calibrate/validate our artificial intelligence based program for estimating grass percentage of mixtures. In 2016 photographs were taken at 1, 2, and 3m heights, using an unmanned aerial system (drone), to assess the potential for drones to be used for these measurements. Multiple types of drones and cameras were tested. Turbulence was excessive at the lower heights, requiring a very high resolution camera for heights of 2m or more. Even with a high resolution camera, this system produced unsatisfactory results. In 2017 over 2000 photos were taken of alfalfa-grass stands. The app tended to overestimate grass percentage at low grass percentages. A correction of the calibration produced estimates that had a 95% confidence interval of ± 12 percentage units of calibrated visual estimates. Additional photos will be taken and evaluated in the spring of 2018 before the app is released for farmer use.

What opportunities for training and professional development has the project provided?
Objective 3. Stakeholders across NY had the opportunity to participate in numerous workshops that discussed alfalfa-grass mixtures and the advantages of higher quality grass and reduced lignin alfalfa in these mixtures. Alfalfa-grass issues were presented and discussed at the Cornell Agricultural Inservice Training for Extension Educators (Nov. 4, 2015), Northeast Regional Certified Crop Advisor Advanced Training (Dec. 1, 2015), the Cornell Field Crop Dealer Meeting for Agricultural Consultants (Nov. 11, 2016), Northeast Regional Certified Crop Advisor Basic Training (Nov. 11, 2016), Northeast Regional Certified Crop Advisor Advanced Training (Nov. 11, 2016), and the New England In-Service Training for Certified Crop Advisors in Portsmouth, NH (two presentations, Jan. 24, 2018).

How have the results been disseminated to communities of interest?
Objective 3. Alfalfa-grass presentations were made at the NY Oneida County Crop Congress (Jan. 6, 2016), NY Southern Tier Crop Congress (Jan. 8, 2016), a series of forage-specific meetings across NY state (Jan. 25, 26, 27, 28, 29, 2016), Northern NY Crop Congress (Feb. 3, 2016), NY Madison County Crop Congress (Mar. 16, 2016), NY Oneida County Crop Congress (Jan. 4, 2017), NY Broome County Crop Conference (Feb. 16, 2017), Western NY Crop Management Conference (Feb. 21, 2017), the Western NY Forage Congress (Feb. 18, 2017), NY Delaware County Crop School (Mar. 1, 2017), NY Steuben County Crop Symposium (Mar. 3, 2017), and the 2017 National Hay Association Conference, Canandaigua, NY (Sept. 30, 2017). One summer field day was held on July 27, 2016 at Curtin Dairy in Oneida County, NY, a facility with 2000 acres of alfalfa-grass. These meetings totaled over 1000 farmer and consultant participants. D.J. Cherney and J.H. Cherney were also the keynote speakers at the Biennial Quebec Forage Conference (Dec. 8-11, 2015), speaking to over 400 farmers and consultants. This NIFA grant was specifically presented at the North American Alfalfa Improvement Conference in Madison, WI (July 13, 2016). An alfalfa-grass abstract was presented at the American Dairy Science Association meetings in Salt Lake City, UT (July 19, 2016), and will be presented at the American Society of Animal Science meetings in Vancouver, BC (July 8-12, 2018). Alfalfa-grass talks will be presented by both J.H. Cherney and D.J. Cherney at the Maryland Cattlemen's Association summer field day at Hedgeapple Farm in Buckeystown, MD (June 23, 2018). Alfalfa-grass articles have been published in Progressive Forage Grower magazine, Progressive Dairyman magazine, The Manager magazine, and the Cornell What's Cropping Up? field crop newsletter.

What do you plan to do during the next reporting period to accomplish the goals?
{Nothing to report}

Participants

Actual FTE's for this Reporting Period

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Student Count by Classification of Instructional Programs (CIP) Code
{NO DATA ENTERED}

Target Audience
Target audiences for this project over the two-year period included farmers and farm advisors, and extension educators as well as state agency personnel.

Products
Citation


Citation


Citation

Product Type
New Germplasm

Description
Assisted in the evaluation and release of two new meadow fescue cultivars, Azov and Hidden Valley.

Product Type
Software or NetWare

Description
Continued development on a cell phone app for evaluating grass percentage in cell phone photographs of alfalfa-grass stands. The artificial intelligence program requires a photo and measurement of current maximum alfalfa height. Products include grass% in mixtures, mixture neutral detergent fiber, and estimated days to harvest at optimum quality. Over 2000 photos collected in 2017 showed a bias in predictions that will be corrected through collection of an additional set of photos in 2018.

Product Type
Data and Research Material

Description
Pure alfalfa and alfalfa-grass studies were established in the spring and late summer in NY, MN, and KY. Data collected included dry matter yield from 3 to 5 harvests per season depending on location, grass percentage of mixtures, and forage quality of separated alfalfa and grass. Forage quality analyses included NDF, ADF, ADL, IVTD, NDFD48h, and CP.

Changes/Problems
Due to poor establishment of the spring-seeded field study in 2016 in MN, late-summer seeded trials were added to this study in NY, MN, and KY. The late-summer seeded trial failed in KY, but was harvested in 2017 in both NY and MN, adding to the data accumulated from spring-seeded trials in NY, MN and KY. Spring and summer seedings will have a final harvest in the spring of 2018, to evaluate yield and grass percentage in mixed alfalfa-grass stands. Since the cell phone app showed some bias for grass percentage in 2017, additional photos will also be taken in the spring of 2018 to re-calibrate the app, which should allow us to release it for farmer use.