V(A). Planned Program (Summary)

Program # 6

1. Name of the Planned Program

Sustainable Energy

☑ Reporting on this Program

V(B). Program Knowledge Area(s)

1. Program Knowledge Areas and Percentage

KA Code	Knowledge Area	%1862 Extension	%1890 Extension	%1862 Research	%1890 Research
201	Plant Genome, Genetics, and Genetic Mechanisms			6%	
203	Plant Biological Efficiency and Abiotic Stresses Affecting Plants			19%	
204	Plant Product Quality and Utility (Preharvest)			62%	
206	Basic Plant Biology			13%	
	Total			100%	

V(C). Planned Program (Inputs)

1. Actual amount of FTE/SYs expended this Program

Year: 2014	Extension		Research	
	1862	1890	1862	1890
Plan	0.0	0.0	2.5	0.0
Actual Paid	0.0	0.0	3.2	0.0
Actual Volunteer	0.0	0.0	0.0	0.0

2. Actual dollars expended in this Program (includes Carryover Funds from previous years)

Extension		Research	
Smith-Lever 3b & 3c	**1890 Extension**	**Hatch**	**Evans-Allen**
0	0	282281	0
1862 Matching	**1890 Matching**	**1862 Matching**	**1890 Matching**
0	0	239852	0
1862 All Other	**1890 All Other**	**1862 All Other**	**1890 All Other**
0	0	0	0

V(D). Planned Program (Activity)

1. Brief description of the Activity

Curly Top Gumweed Biofuel Potential
The fourth year of biomass field trials is completed. The team has work this past year on improved germination rates protocols, with some methods yielding 85% success rates in greenhouses. Fertilization trials in combination with irrigation trials were conducted in 2014. The team has developed a harvesting protocol that employees a two cutting strategy. Another accomplishment was working out the protocols to decolorize grindelic acid, a bio-crude extracted derived from gumweed.

Improving Camelina as a Biofuel Alternative
Varietal trials with eight named varieties of Camelina (e.g., Blaine Creek, Calena, Celine, Cheyenne, Columbia, Ligena, Suneson, and Yellowstone) were conducted again during the spring of 2014. The team analyzed data for biomass production (aerial, body, and root) and seed production.

Ethylmethane sulfonate (EMS) mutant populations of Camelina sativa were produced as a major output of this project and continue to undergo screening on a continuing basis. To date, seeds have been collected for a total of 5,243 second generation mutant (M2) lines from a total of ~7,000 M2 lines collected. M2 lines were replanted and 1,568 third generation (M3) lines have been grown out. Of these, 353 M3 lines were dried and waiting seed cleaning; 1215 M3 lines have been cleaned. A total of 474 M3 lines were screened for mucilage defect using the Ruthenium red staining protocol. One mucilage-fee line (Cs98) was isolated and found to exhibit a stable mucilage-free defect in the M4-M6 generations. A total of 250 M3 lines have been screened for altered fatty acid methyl ester composition using gas chromatography to isolate mutants with high oleic acid and low uric acid content.

Transformation trials using the hygromycin selectable marker gene are in progress for several target genes of interest. Efforts have focused on the herbicide and drought tolerance traits during the current reporting period. A total of 23 independent glyphosate resistant T2 Camelina sativa lines have been generated of which nine have been confirmed in the T3 generation to be tolerant to glyphosate herbicide.

A massively parallel (454) pyrosequencing was completed to characterize the transcriptome of Camelina sativa in order to identify major genetic determinants responsible for stress-adaptive responses.

Accumulation of Hydrocarbons in Plants - making biofuels
The long-term goal is to develop a technology to increase the accumulation of hydrocarbons in a Nevada biofuel crop. Current efforts to produce mid-chain alkanes in Arabidopsis thaliana and Yarrowia lipolytica (a high oil content yeast) have consisted of overexpressing the cyanobacteria derived alkane biosynthesis enzymes Acyl-ACP Reductase and Aldehyde Deformylating Oxygenase.

Prickly Pear Cactus Feedstock
Transcriptomic sequencing using Illumina RNA-sequencing technology was completed during the previous reporting period. The team has developed methods for creating very high quality, transcriptome assemblies from these Illumina datasets using the Evidential Gene pipeline to deal with polyploidy nature of this cactus. A modified transformation protocol has been developed and is currently undergoing testing.

Field trails are now underway in southern Nevada. Three species of Prickly Pears (Indian fig, Pancake, & Nopal Cardón) were propagated in the greenhouse and transplanted to the field. An irrigation system was installed and rabbit fencing was installed around the perimeter of the field site to prevent herbivory by local mammalian fauna (i.e. gophers, and rabbits).

2. Brief description of the target audience

Those most affected by this research include the general public, fellow scientists, energy and chemical industry, and potential producers.

3. How was eXtension used?

The project has one extension Co-PI on the project, as well as, assisting in the maintenance of prickly pear cactus field trial plots.

V(E). Planned Program (Outputs)

1. Standard output measures

2014	Direct Contacts Adults	Indirect Contacts Adults	Direct Contacts Youth	Indirect Contacts Youth
Actual	0	0	0	0

2. Number of Patent Applications Submitted (Standard Research Output)

Patent Applications Submitted

Year: 2014

Actual: 0

Patents listed

3. Publications (Standard General Output Measure)

Number of Peer Reviewed Publications

2014	Extension	Research	Total
Actual	0	5	0

V(F). State Defined Outputs

Output Target

Output #1

Output Measure

- Peer reviewed journal articles, publications in commodity group publications, presentations at scientific meetings, presentations at stakeholder, Native American and agency meetings.
  Not reporting on this Output for this Annual Report

Output #2

Output Measure

- Demonstrations and Field Days Conducted

Year	Actual
2014	2

Output #3

Output Measure

- Number of Graduate Students or Post-Doctorates Trained

Year	Actual
2014	11

Output #4

Output Measure

- Number of Undergraduate Students Involved in Research

Year	Actual
2014	20

Output #5

Output Measure

- Research Projects Conducted

Year	Actual
2014	0

Output #6

Output Measure

- Leveraged Research Projects

Year	Actual
2014	56000

Output #7

Output Measure

- Web Sites Created or Updated

Year	Actual
2014	1

Output #8

Output Measure

- Presentations

Year	Actual
2014	23

Output #9

Output Measure

- Databases, Models, Protocols, and Research Materials

Year	Actual
2014	2

Output #10

Output Measure

- Peer reviewed journal articles, chapters, or books

Year	Actual
2014	6

V(G). State Defined Outcomes

V. State Defined Outcomes Table of Content

O. No.	OUTCOME NAME
1	New fundamental or Applied knowledge
2	Improved skills of students, post-doctorate, and stakeholders
3	New or improved methods
4	Adopt and use new methods or improved technology
5	Use new or improved plant varieties
6	Increase economic competitiveness
7	Improve productivity in a semi-arid environment
8	Adopt new improved skills
9	Developing Biofuel Feedstock Tailored to Nevada's Arid Climate

Outcome #1

1. **Outcome Measures**

   New fundamental or Applied knowledge

   Not Reporting on this Outcome Measure

Outcome #2

1. **Outcome Measures**

   Improved skills of students, post-doctorate, and stakeholders

   Not Reporting on this Outcome Measure

Outcome #3

1. **Outcome Measures**

   New or improved methods

   Not Reporting on this Outcome Measure

Outcome #4

1. **Outcome Measures**

   Adopt and use new methods or improved technology

   Not Reporting on this Outcome Measure

Outcome #5

1. **Outcome Measures**

   Use new or improved plant varieties

   Not Reporting on this Outcome Measure

Outcome #6

1. **Outcome Measures**

   Increase economic competitiveness

   Not Reporting on this Outcome Measure

Outcome #7

1. **Outcome Measures**

   Improve productivity in a semi-arid environment

   Not Reporting on this Outcome Measure

Outcome #8

1. **Outcome Measures**

   Adopt new improved skills

   Not Reporting on this Outcome Measure

Outcome #9

1. **Outcome Measures**

   Developing Biofuel Feedstock Tailored to Nevada's Arid Climate

2. **Associated Institution Types**

   - 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Actual
2014	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
The long-term goal of the proposed research is to develop a number of drought tolerant species (e.g., curly top gumweed, camelina, and prickly pear cactus) into new biofuels and feed crops adapted to our arid western region. Our vision is to create novel biofuel feedstock production platforms with increased energy density within vegetative tissues that is capable of producing biodiesel, renewable diesel, or jet fuel with minimal water inputs required for traditional biofuel feedstocks.

What has been done
The research efforts conducted over the past year are the culmination of numerous activities. In the lab, bacterial enzymes have been introduced into two model plants (mouse-ear cress and Yarrow) to boost the hydrocarbon production. Mutant populations of Camelina (aka, wild flax) were produced that are free of gelatinous mucilage well into the sixth generation of propagation. Third generation mutants were also screened for a suite of promising traits. This includes altered fatty acid compositions, rapid completion of ontology, shade tolerance, and greater plant densities. Addition work was conducted on Camelina mutants tolerant to glyphosate herbicides.

Field and greenhouse varietal trials are well underway for Camelina, Curly Top Gumweed, and Prickly Pear Cactus.

Results
Based upon Camelina field trials for seed production and oil content, our scientist are now recommending that producers of northern Nevada go with Celine, Columbia or Suneson varieties. Research into transgenic lines of Camelina has increase seed size by 40%, which translates into high oil content per seed. This line of research has also created a rapid cycler variety that should serve as an outstanding cover crop.

The biofuels team has created a gas exchange apparatus in collaboration with local manufacturer Tripp Plastics that will assist scientists interested in measuring gases produced during Prickly Pear Cactus development.

Finally, an interactive educational display was created (Bio-Energy Crops for Semi-Arid Environment) that introduces participants to common biofuel feedstocks found in Nevada and the oils produced.

4. Associated Knowledge Areas

KA Code	Knowledge Area
201	Plant Genome, Genetics, and Genetic Mechanisms
203	Plant Biological Efficiency and Abiotic Stresses Affecting Plants
204	Plant Product Quality and Utility (Preharvest)
206	Basic Plant Biology

V(H). Planned Program (External Factors)

External factors which affected outcomes

- Economy
- Appropriations changes

Brief Explanation

DNA extraction from Prickly Pear Cactus was extremely challenging due to the high pectin content of the cladodes. Numerous DNA extraction methods were attempted before a successful method was discovered. This resulted in delays in the isolation of high-quality DNA. Once again, Nevada annual average precipitation continues to decline, with the past three winters scoring some of the driest on record. Without adequate rainfall, work on gumweed and prickly pear cactus may require alternative sources of water.

V(I). Planned Program (Evaluation Studies)

Evaluation Results

The germination rates of Curly Top Gumweed has been improved to 80-85% through improved protocols.

The discovery of a DNA extraction method has resulted in the isolation of high-quality genomic DNA. A critical success of the project to allow for the isolation and characterization of native promoters found in Prickly Pear Cactus.

Varietal trials on Camelina has uncovered a rapid cycling variety that would server well as a fall cover crop.

Key Items of Evaluation

- Bioenergy Crops for Semi-arid Environments" educational display was created
- 23 independent glyphosate resistant second generation (T2) Camelina sativa lines were generated of which nine have been confirmed in the T3 generation to be tolerant to glyphosate herbicide.
- A transcriptome was created to identify major genetic determinants responsible for stress-adaptive responses in Camelina.
- A line of mucilage-fee Camelina (Cs98) was isolated and found to exhibit a stable mucilage-free defect in the M4-M6 generations.
- A DNA extraction method for Prickly Pear Cactus was discovered that gets around the high levels of pectin found in the cladodes (leaves).