PROJECT STATUS UPDATE

SBAR Retreat
University of Arizona, Tucson AZ
11-13 September 2019

FEEDSTOCK DEVELOPMENT & PRODUCTION

***LEADS**: Dennis Ray & Pete Waller*

***Team**: Abdel-Haleem, Angadi, Brown, Chen, Cruz, Didan, Dierig, Dittmar, El-Shikha, Evancho, Garcia, Grover, Gunatilaka, Heinitz, Hoare, Holguin, Hunsaker, Katterman, Madasu, Maier, Maqsood, McCloskey, McMahan, Neilson, Ogden, Pastor, Pradyawong, Rogstad, Schmalzel, Teetor, Wang, Willmon, Xu*

SBAR

USDA United States Department of Agriculture National Institute of Food and Agriculture

Feedstock Development and Production

- Objective 1: Improve biomass quantity and quality through genetics and traditional breeding.
  - *Objective 1a: Exploitation of Apomixis - Guayule*

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Guayule Yield Tests

Traditional breeding is dependent upon having available genetic resources (germplasm) from which to identify useful traits.

Established (Maricopa, Eloy, Tucson) April 2018 (now 17 months of age)

Final harvest will be April 2020 (2 years of age)

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Accomplishments and PIs

- Phenotypic data for one-year
- Biomass, rubber, and resin data for one-year
- Cultivation practices for each location one-year

- Hussein Abdel-Haleem (USDA)
- David Dierig (Bridgestone)
- Dennis Ray (U of A)

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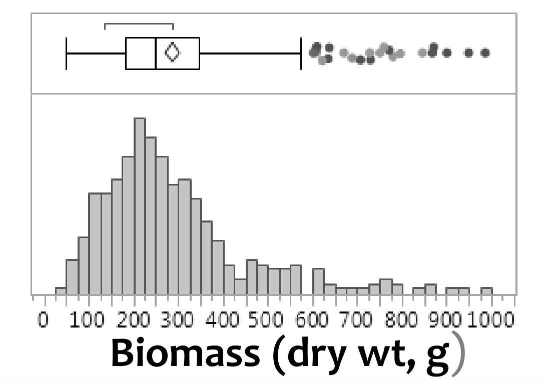
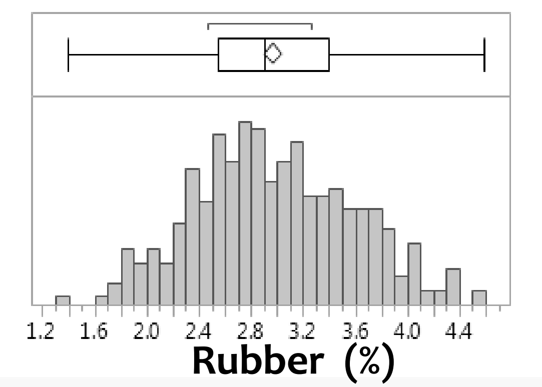
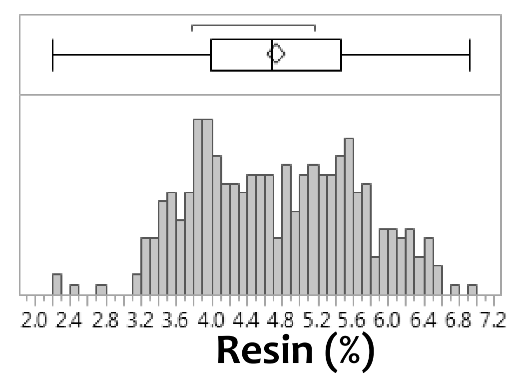
Field and Lab Data

- Height
- Width
- Biomass
- Flowering time



- Rubber and Resin





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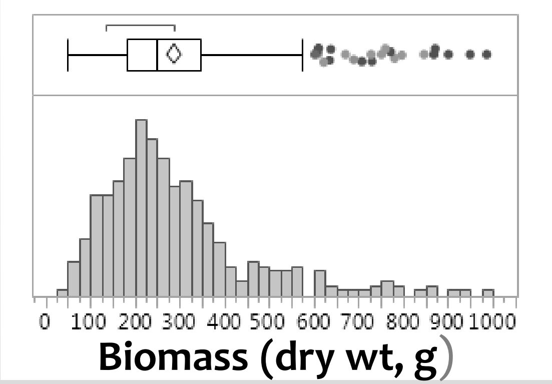
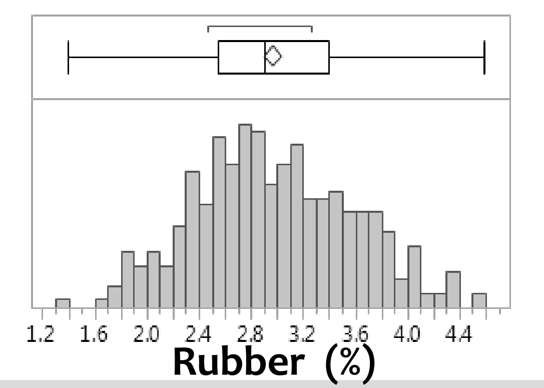
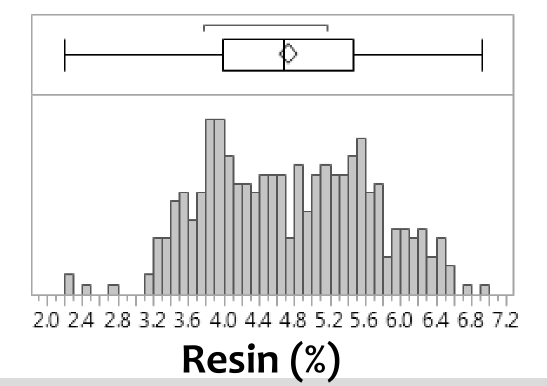
Feedstock Accomplishments (Bridgestone)

Variety Trial of USDA lines – Year 2

- **Variation in seed weight among accessions determined**
- Utilized high throughput phenotyping method to track growth and development
- **Diversity within and among public guayule accessions was estimated based on ploidy of 1,493 plants analyzed (11619 and N565II had highest diversity)**
- Groups of desirable germplasm for feedstock improvement were identified based on biomass, rubber and resin content (11 months) and will be compared after 24 months (harvest period)







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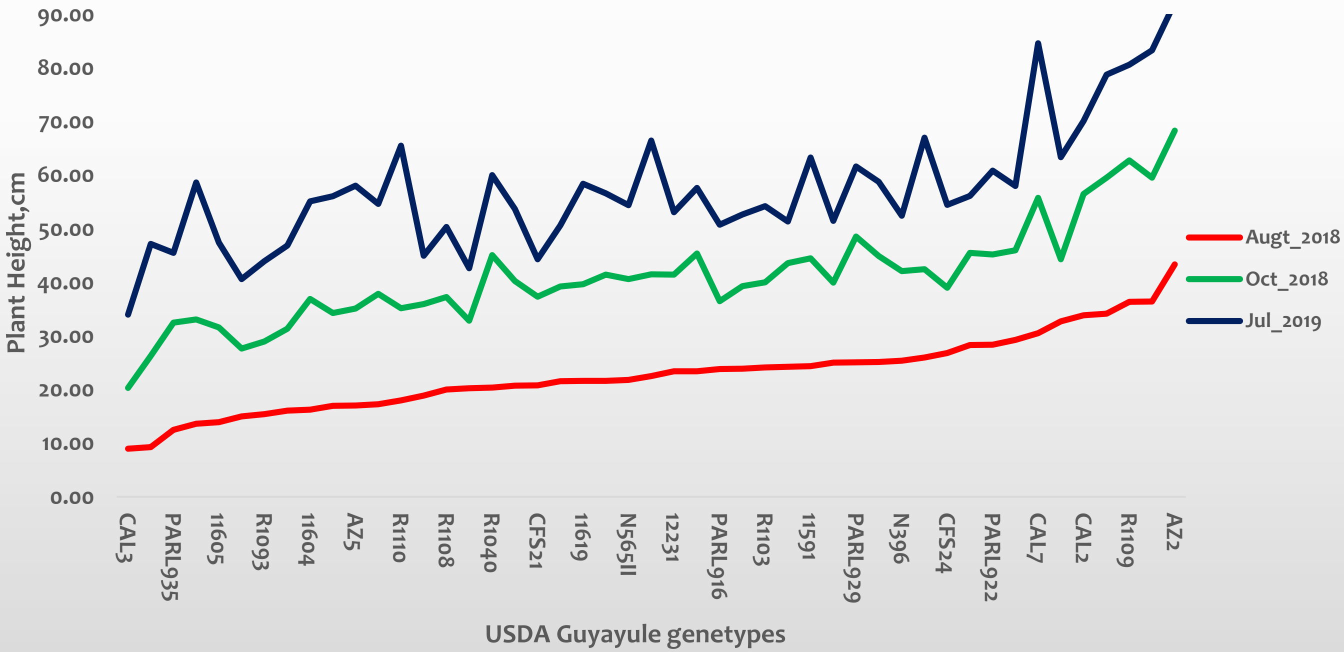
Feedstock Accomplishments 2019 (USDA-ARS, Maricopa)

Hussein, **Greg Leake, Lily Luo, Aaron Szczepanek , Matt Conley,**
Adrianna Chambers, Amber Dearstyne, Brandon Vera

Field phenotyping of one-year old guayule genotypes



- **There are phenotypic variations within USDA population**
- **Tetraploid genotypes such as AZ-2 were taller than the diploid (CAL-3)**

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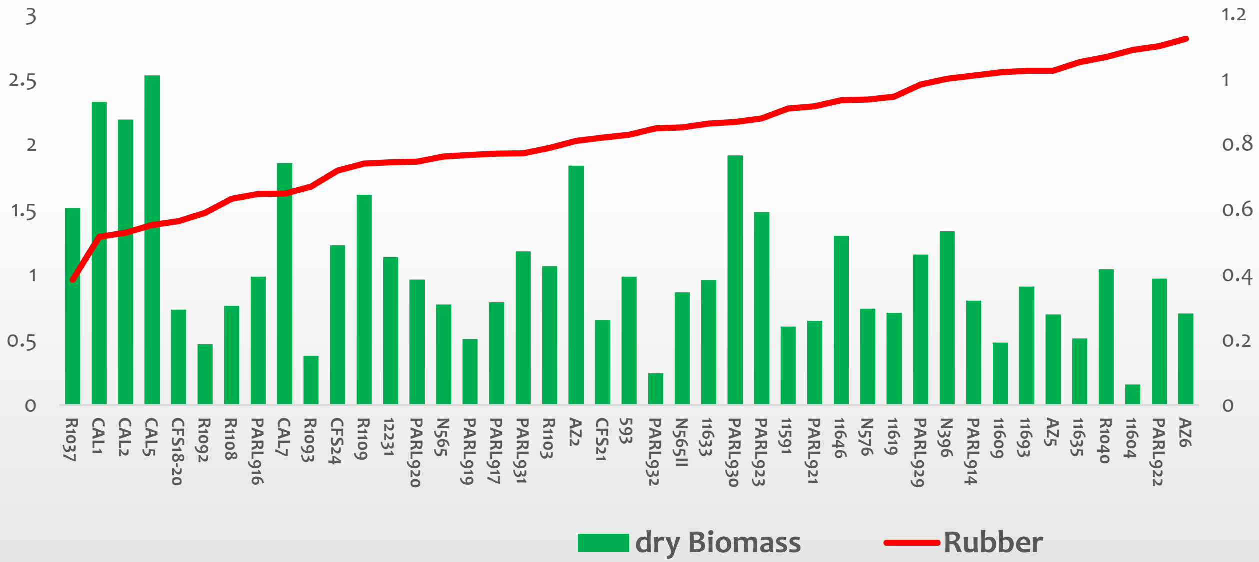
There are phenotypic variations within guayule for rubber and biomass

Lab phenotyping of one-year old plants guayule genotypes



- Improved germplasm tend to be higher in rubber yield with greater biomass
- Negative correlation between biomass and rubber content

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Key Point

- Desirable germplasm for feedstock improvement were identified based on biomass, rubber content, and resin content (12 months), and will be compared after 24 months (harvest period)

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Year 3

- Continue collecting and analyzing field/lab phenotypic data of two-year old guayule plants (plant height, biomass, flowering time, rubber and resin) (All locations)
- Study the stress responses and stability of guayule genotypes (MAC)
- Cataloging of guayule leaf wax classes produced in response to stress condition (MAC)
- Final harvest of yield test (All locations)
- Share production and yield data with other researchers on the grant (All locations)

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Feedstock Development and Production

- Objective 2: Develop high-throughput phenotyping to support crop expansion using remote-sensing methods to create interactive databases/tools.

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Accomplishments and PIs

- **Monitoring** plant growth/health using NDVI.
- **Detection** of weeds at early stages.
- **Correlating** remote sensing to field data such as plant height, %cover, plant moisture, rubber/resin content and yields.
- **Correlating** remote sensing data to crop coefficient.
  - **Predict** crop coefficient for different locations, environments, and planting seasons
- **Correlating** RGB and multispectral vegetation indices.

- Hussein Abdel-Haleem (USDA)
- David Dierig (Bridgestone)
- Diaa El-Shikha (U of A)
- Peter Waller (U of A)

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Crop Circle ACS-470 active sensor data

- **A high clearance tractor carrying two crop circle ACS-470 active sensor (Holland Scientific Inc., Lincoln, NE) was used to collect data**:
  - *The first has filters of 800 nm (NIR-20 nm bandwidth), 590 nm (Amber-10 nm bandwidth), and 670 nm (Red-10 nm bandwidth).*
  - *The second sensor had filters of 780 nm (NIR-20 nm bandwidth), 530 nm (Green-10 nm bandwidth), and 730 nm (RE-10 nm bandwidth).*
- **Canopy reflectance was used to calculate the normalized difference vegetation index (NDVI).**

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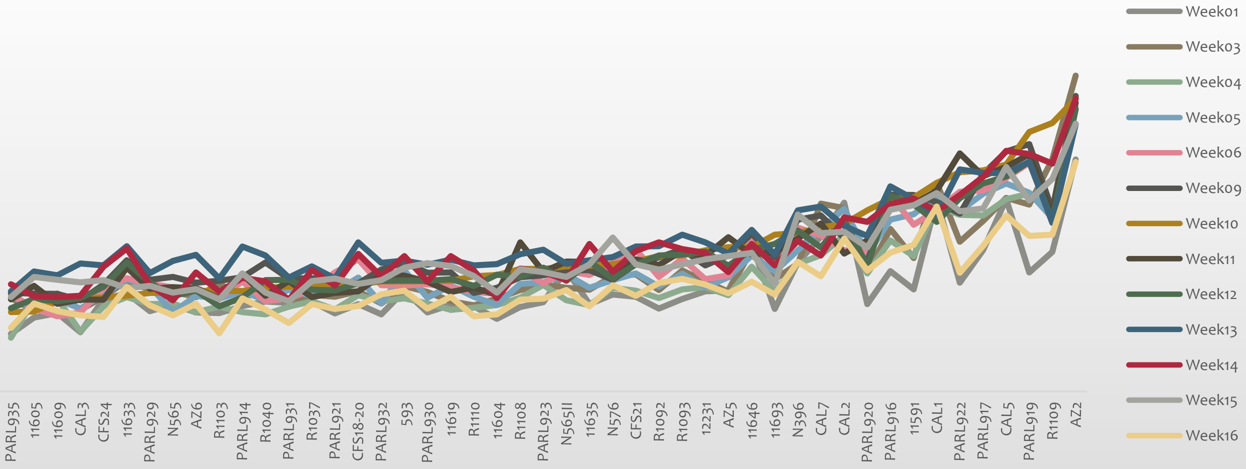
High-throughput phenotyping and remote sensing of one-year old guayule genotypes

Variations in NDRE, a vegetation index, among guayule genotypes and weeks

- **Remote sensing could be used to detect the phenotypic variations among guayule plants**
- **Improved germplasm tend to be greener compared to wild**



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Multispectral data

RGB data

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Feedstock Accomplishments to gain
2020
(USDA-ARS, Maricopa)

- Continue collecting and analyzing field/lab phenotypic data of two-year old guayule under well irrigated conditions (plant height, biomass, flowering time, rubber and resin, and HTP-related traits)
- Study the stress responses and stability of guayule collection and genotypes
- Cataloging of guayule leaf wax classes produced in response to stress conditions




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Feedstock Development and Production

- **Objective 1: Improve biomass quantity and quality through genetics and traditional breeding.**
  - ***Objective 1b: Flowering to improve yield – guayule.***

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Accomplishments and PIs

- Constructs prepared; transformations underway
- Target genes = transcription factors related to flowering in plants
- Analysis of gene expression from field plants to identify bioengineering targets

- Colleen McMahan (USDA)
- Julie Nelson (U of A)
- Duke Pauli (U of A)

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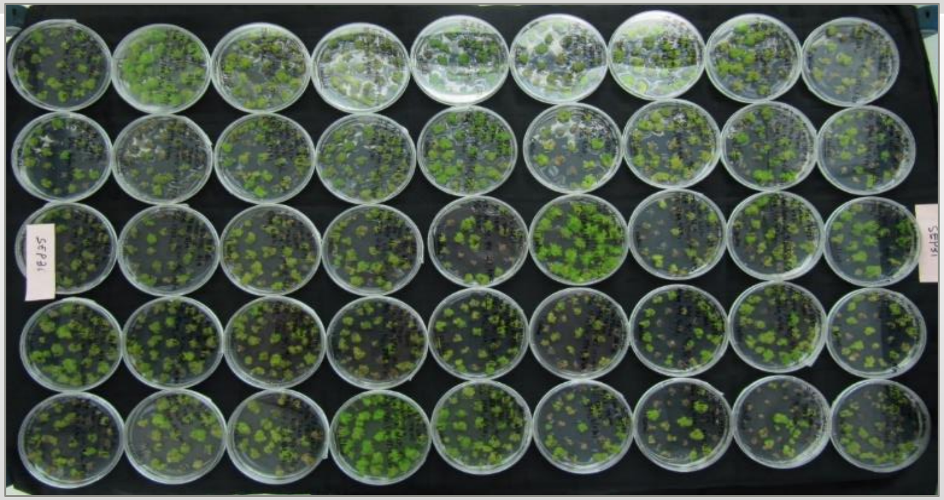
FEEDSTOCK DEVELOPMENT & PRODUCTION
USDA-ARS Rubber Lab– Colleen McMahan

Obj 1: Improve biomass quantity and quality through genetics and traditional breeding
Sub Obj: 2) Downregulate flowering to improve yield - guayule

Constructs prepared; transformations underway Target genes=transcription factors related to flowering in plants	
APETALA1	~350 calli/shoots growing in culture
SEPATALLA3	~1890 calli/shoots growing in culture
FLOWERING LOCUS T	~306 calli growing in culture
LEAFY	Transformations underway
False positives are being eliminated through selection rounds.	



SEED summer intern Milagrom Adom
SBAR technician Mariano Resendiz

Team: Niu Dong, Trinh Huynh, Mariano Resendiz, Dante Placido, Grisel Ponciano

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FEEDSTOCK DEVELOPMENT & PRODUCTION
USDA-ARS Rubber Lab (McMahan Lab), UA (Pauli, Nelson)

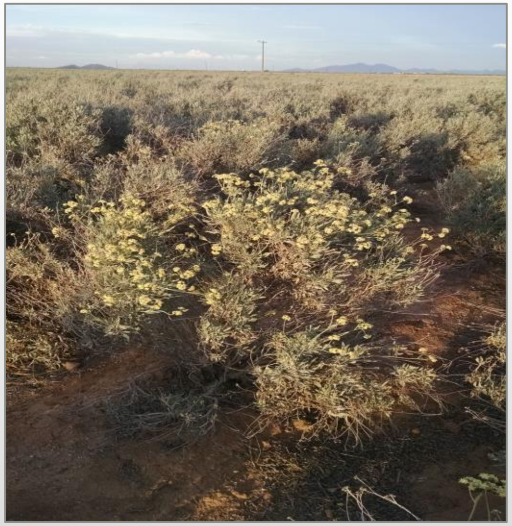
United States Department of Agriculture
National Institute of Food and Agriculture

Obj 1: Improve biomass quantity and quality through genetics and traditional breeding
Sub Obj: 2) **Downregulate flowering to improve yield - guayule**

- *Hypothesis: Genetic modification of guayule to reduce/eliminate flowering might increase rubber content (based on results from Willard 1985)*
- ***Strategy: Analysis of gene expression from field plants might identify bioengineering targets***

Hunsaker, El-Shikha et al. 2019 irrigation trial Stem tissue collected for gene expression studies	
Control (100% ETR)	Drought (25% ETR)
No flowering	Flowering
5.97 % rubber content	8.58 % rubber content

Drought-induced flowering was associated with higher % rubber.
SBAR studies will clarify these contradictions.



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FEEDSTOCK DEVELOPMENT & PRODUCTION
USDA-ARS Rubber Lab (McMahan Lab), UA (Pauli, Nelson)

Obj 1: Improve biomass quantity and quality through genetics and traditional breeding
Sub Obj: 2) Downregulate flowering to improve yield - guayule

- *Hypothesis: Genetic modification of guayule to reduce/eliminate flowering might increase rubber content (based on results from Willard 1985)*
- ***Strategy: Analysis of gene expression from field plants might identify bioengineering targets***

- ***Results: (Nelson et al. submitted)***
  - **13 of the candidate genes identified with differential expression impact flowering (~46%).**
  - **Data represent the first genomic analyses of how guayule responds to drought-like conditions in agricultural production settings.**
  - **Identified a number of regulators of abiotic responses, including transcription factors and lncRNAs, that are strong candidates for modulating rubber biosynthesis under water-limiting conditions common to guayules' native production environment.**
- ***Additional analyses focus on gene expression and rubber biosynthesis (Dong et al. poster)***

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FEEDSTOCK DEVELOPMENT & PRODUCTION
USDA-ARS Rubber Lab (McMahan Lab)
Key Tasks for Year 3

- ***Sub Obj: 2) Downregulate flowering to improve yield - guayule***
  - **Finalize 2 publications on gene expression of flowering/non-flowering plants.**
  - **Continue additional transformations (LEAFY) until Dec 2019.**
  - **Recover plants and evaluate genotypes (DNA, RNA) and phenotypes (rubber content, plant architecture, etc.) through Year 3.**

- *Obj. 5. Develop soil quality and health knowledge critical to environmental sustainability.*
  - Complete data analysis of physiological and chemical characte of samples collected during Year 2 winter dormancy growth s with Julia Neilson.

    Plant architecture, carbon fixation rate, gene expression, rubb biosynthesis rate, rubber particle and molecule characteristics

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Feedstock Development and Production

- **Objective 3: Deploy superior genotypes of guayule and guar to regional growers.**

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Guar Yield Tests

30 germplasm lines evaluated at three locations.

Superior genotypes will be identified for each location.

Established June 2019

Harvest October/November 2019

Clovis, NM

Las Cruces, NM

Tucson, AZ

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Accomplishments and PIs

- Sangamesh Angadi (NMSU)
- Kulbhushan Grover (NMSU)
- Dennis Ray (U of A)

- Germplasm lines increased and distributed to three sites
- Yield tests established
- Field performance of genotypes being evaluated

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Guar Breeding

- Evaluating diverse germplasm
  - *Germplasm and released lines*
  - *Generating new genetic diversity by crossing to male-sterile lines*

Composite crosses

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Guar research - Year 3

- **Feedstock Development**
  - ***Evaluate field performance of Guar genotypes (All locations).***
- **Production technology**
  - ***Evaluate response of guar to planting densities (Las Cruces).***
- Scientific interactions
  - *Deliver presentations at scientific meetings.*

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Feedstock Development and Production

- Objective 4: Deploy agronomic production practices; identify agronomic information for salinity, herbicide, and nutrients to support production; provide irrigation apps using algorithms to growers.

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PIs

- Hussein Abdel-Haleem (USDA)
- Sangamesh Angadi (NMSU)
- David Dierig (Bridgestone)
- Diaa El-Shikha (U of A)
- Kulbhushan Grover (NMSU)
- William McCloskey (U of A)
- Dennis Ray (U of A)
- Peter Waller (U of A)

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Guar accomplishments- Year 2

- Feedstock Development
  - *Guar germplasm multiplied.*
  - *Germplasm evaluation started.*
- **Production technology**
  - ***Guar moisture stress study completed (Las Cruces).***
- Scientific interactions
  - *Presentations delivered at scientific meetings.*

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Co-PI: Sangu Angadi

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National Institute of Food and Agriculture

Title: Pre-irrigation and critical growth stage based irrigation to improve water efficiency of guar

Accomplishments:

- Guar, unlike many annual crops, tolerates skipping irrigation after flowering.
- Pre-irrigation of guar significantly increased seed yield. This gives an opportunity to irrigate during cooler, off season, without competition from traditional cash crops.
- Among seed components, pods per plant was more sensitive to water stress.

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Co-PI: Sangu Angadi Jagdeep Singh (GS)

Title: Pre-irrigation and critical growth stage based irrigation to improve water efficiency of guar

Table 1. Effect of different water availabilities on yield parameters of guar crop

Treatments	Seed yield (kg/ha)	Branches plant^{-1}	Pods plant^{-1}	Seed pod^{-1}	1000 seed wt(g)	HI (%)
Pre	1024.0 a	4.9 a	62.9 a	3.6 a	23.4 b	30.8 a
No-Pre	807.2 b	4.0 b	47.8 a	3.8 a	28.8 a	35.8 a
Critical growth stage based irrigation						
Irr	982.5 a	5.0 a	64.2 a	3.9 a	25.9 ab	29.1 c
Rst	976.5 a	4.8 ab	62.3 a	3.8 a	25.6 ab	34.3 ab
Vst	810.8 b	4.1 bc	47.7 b	3.5 a	24.1 b	32.3 bc
Rainfed	892.8 ab	4.0 c	47.2 b	3.8 a	28.7 a	37.4 a
Cultivars						
Kinman	955.6 a	6.4 a	57.5a	3.5 b	27.7 a	32.0 a
Monument	875.7 b	2.5 b	53.2 a	4.0 a	24.5 b	34.6 a

Location: Agricultural Science Center at Clovis.
Irrigation: Center pivot irrigation method
Cultivars: Kinman (branching)
Monument (non-branching)
Year: 2018 and 2019

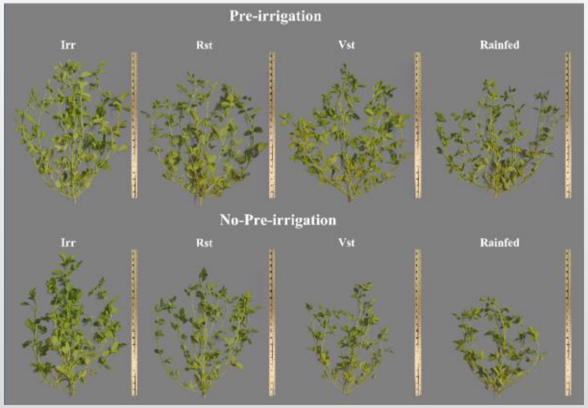
Objectives: Develop deficit irrigation management strategies for guar production in the High Plains.

Main Treatments:

Pre-irrigation (127 mm) (Pre)
No-Pre-Irrigation (0 mm) (No-pre)

Sub Plots:

Irrigated (198 mm) (Irr)
Stress after flowering (71 mm) (Rst)
Stress before flowering (150 mm) (Vst)
Rainfed (23 mm) (Rst)



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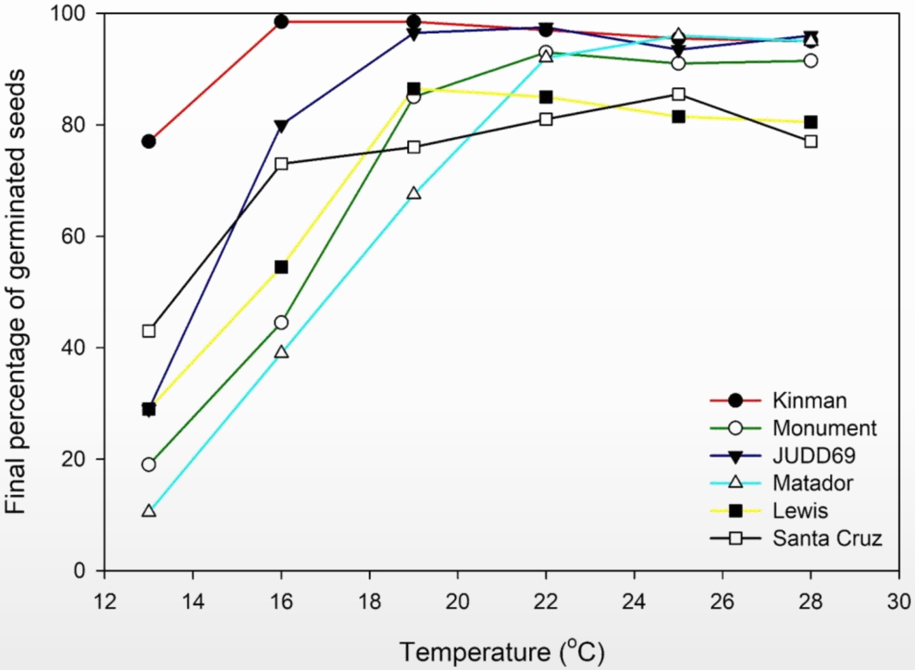
Co-PI: Sangu Angadi and Jagdeep Singh (GS)

Title: Temperature requirements for different guar cultivars for germination



Objectives: Assess genetic variation for optimum temperature for germination in current guar cultivars.

- Guar cultivars varied in temperature requirement for germination. Kinman was the best cultivar under low temperature.
- **If planting in northern latitude or trying earlier planting then Kinman and Judd69 were the best.**

Title: Assessing USDA Germplasm in East Central New Mexico

Objectives: Identify better guar germplasm for adoptability at northern latitude and higher altitude.

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Co-PI: Sangu Angadi

Title: Tasks for September 2019 to August 2020

- **Guar Galactomannan Assay:** To assess water stress effect on galactomannan content of two guar cultivars. Seeds from deficit irrigation studies will be used.
- **Deficit Irrigation Management Study**: Collect data from second year study and initiate 2020 study.
- **Guar USDA Germplasm Evaluation:** Collect data from 2019 season and repeat the study in 2020.
- **Assess Genetic Variation in Guar Germplasm for Germination Temperature Requirement.**

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Guayule Irrigation Questions Asked

- **1. How is growth and rubber/resin content affected by location, irrigation amount, or irrigation method over time?**
- **2. Is production better on a particular soil type?**
- **3. What are the benefits of either soil type?**

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Methods

- **DOP: April 2018 at Eloy, AZ (clay soil) and Maricopa, (sandy loam soil).**
- Guayule was direct seeded using AZ-2
- Treatments: Eloy- 100 % and 50 % subsurface drip (SDI) and flood. Maricopa- same except the 50% flood was not included there.
- 1 m plots were harvested at 4, 6, 8,10, 12, 14, and 16 months-old from three replicates of each treatment. Plants were cut at ground level and roots were dug to obtain as much of the main taproot as possible.
- Measurements: 1. # plts/m^2. 2. Root length 3. Fresh and dry weights. 4. separating leaves, stems, roots, and flowers. 5. Rubber and resin content. 6. Leaf samples (later analysis) 7. Seed collection (later analysis)

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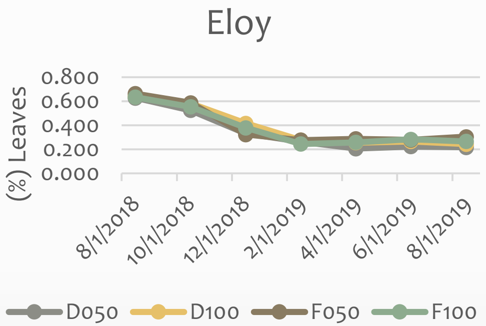
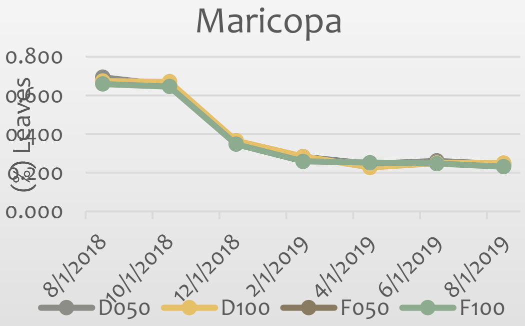
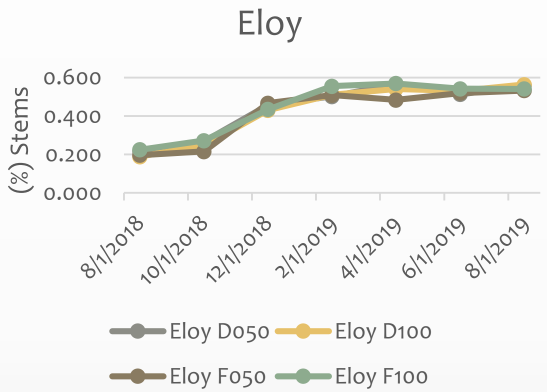
Proportion of Leaves, Stems, and Flowers over time

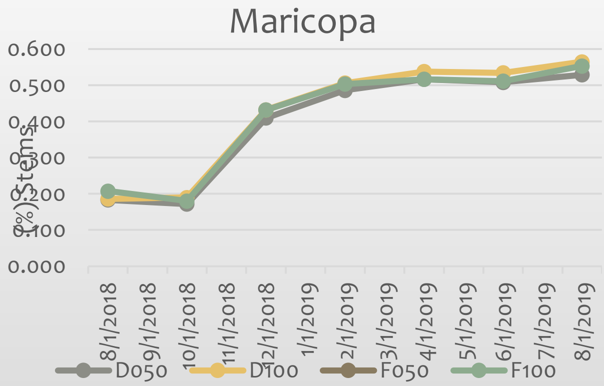
Leaves



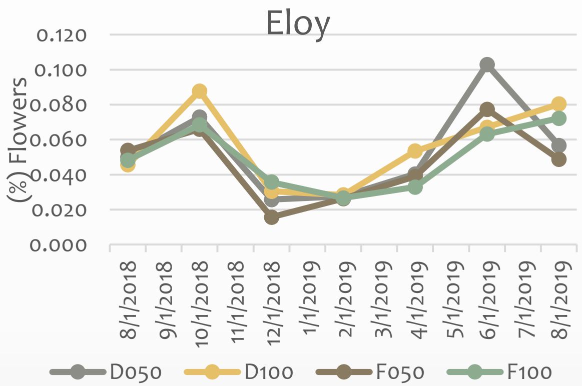


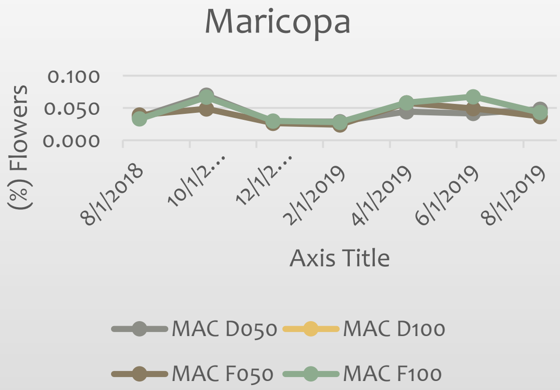
Stems





Flowers





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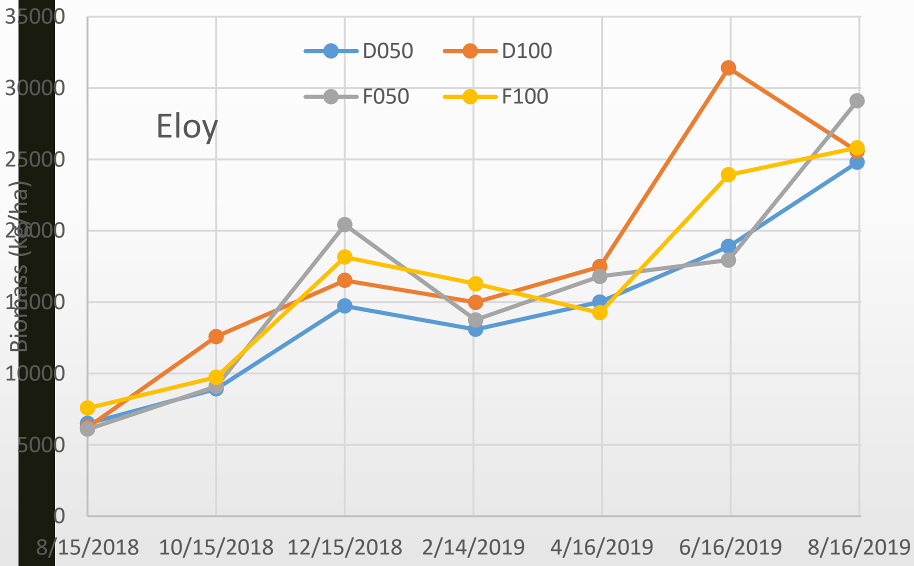
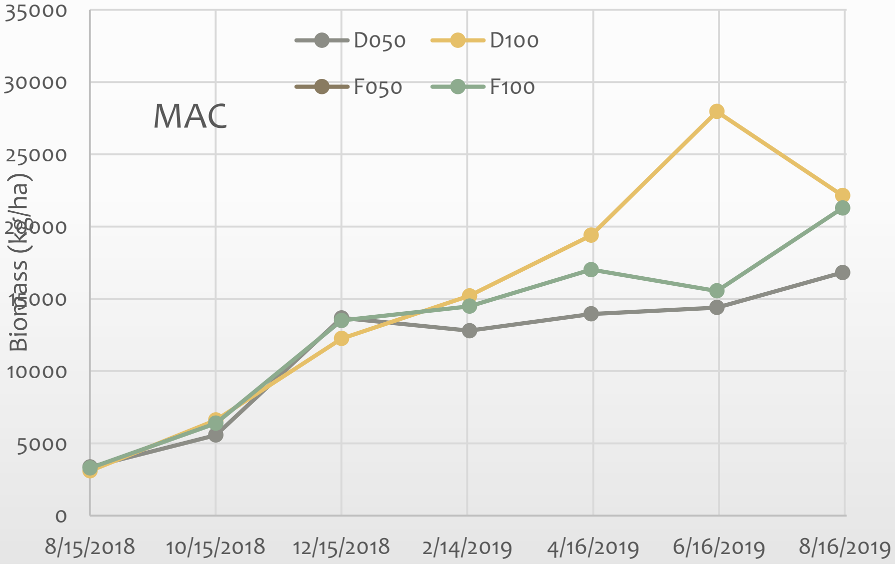
Biomass Accumulation





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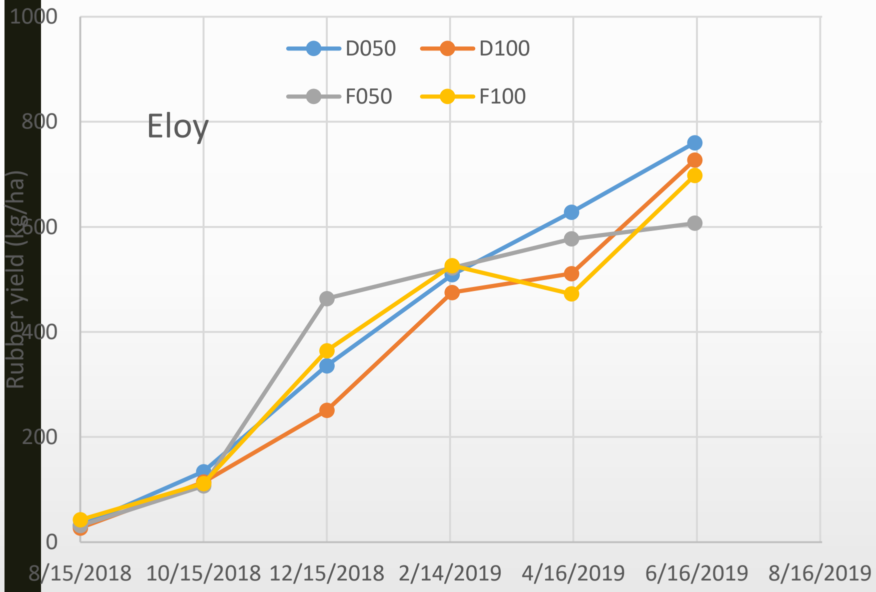
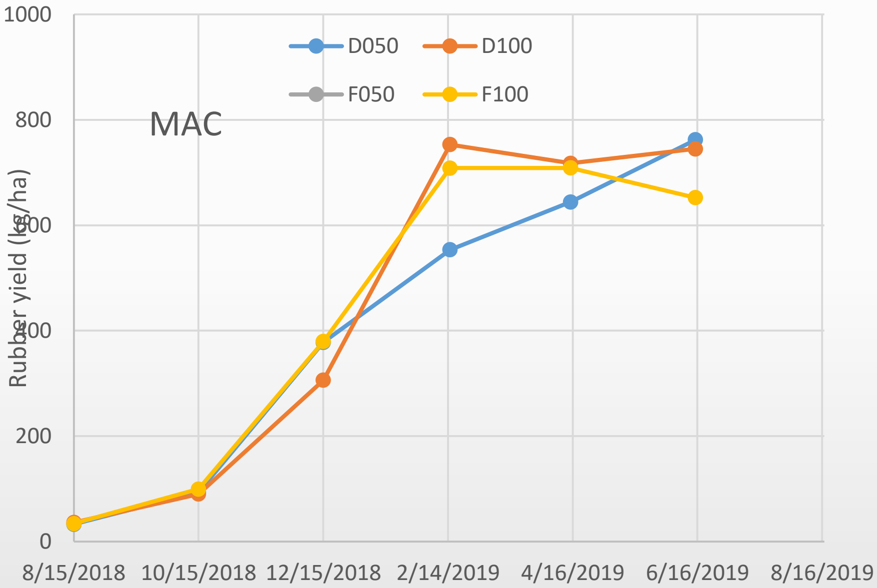
Rubber Yield over time





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Conclusions

1. The proportion of leaves and stems is very consistent over the growth cycle but the ratio changes by 10-months-old from 65% leaves:25% stems to 25% leaves:65%.

Flowers are dependent on the long day photoperiod and temperature.

- The irrigation treatments affected the amount of flowering

- Biomass accumulation was higher at Eloy compared to Maricopa. The 50% drip treatment was lower than other treatments at both locations. At Eloy, the 50% drip and the 100% drip and flood treatments were similar by August 2019 (16 months).

- All treatments produced acceptable biomass yields by 16 months.

- Resin content was prevalent in near-full quantities prior to cold induction at 6- and 8-months of age.

- Rubber content was higher at Maricopa compared to Eloy, perhaps related the water stress mechanism from sandy soils.

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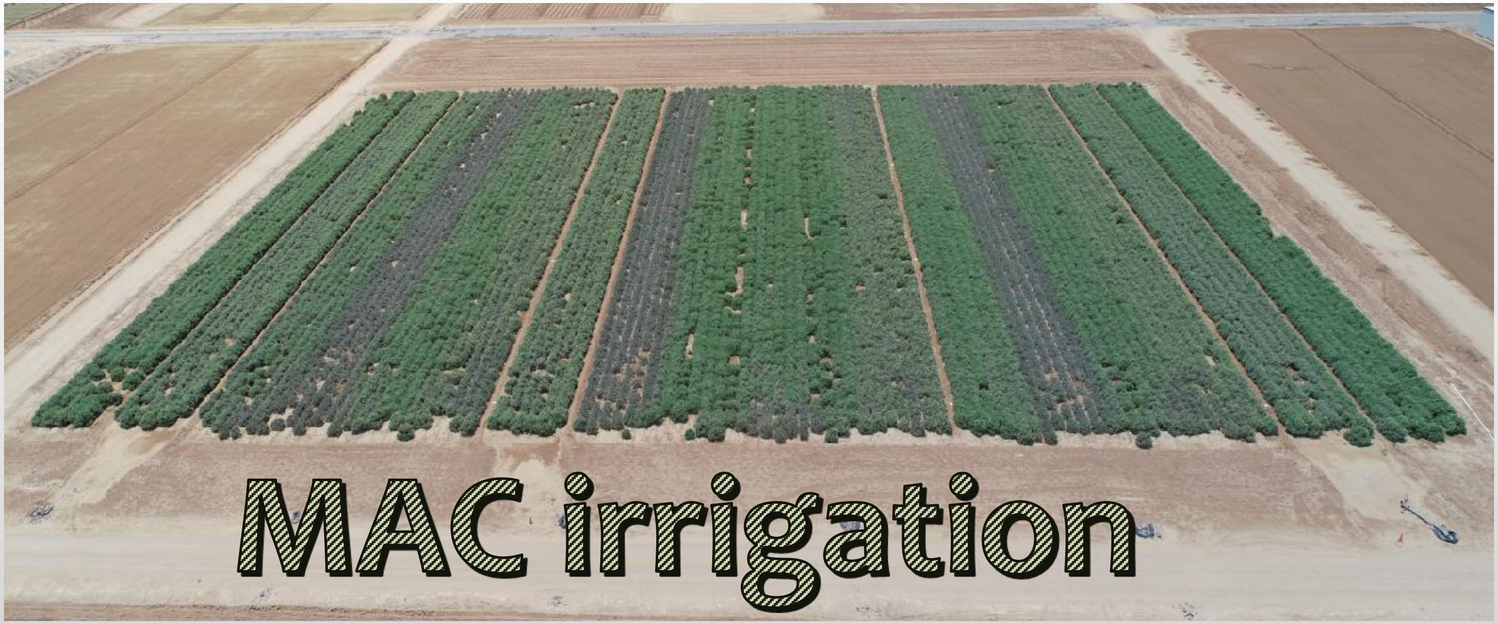
Growing direct-seeded guayule with furrow and subsurface drip irrigation

- MAC and Eloy
  - *Irrigation, Field and soil data, biomass sampling, rubber and resin contents in 6 irrigation treatments (5 drip and 1 flood)*
  - ***Remote sensing images (RGB and multispectral) correlated with measured cover and correlated with irrigation requirements (Kc).***



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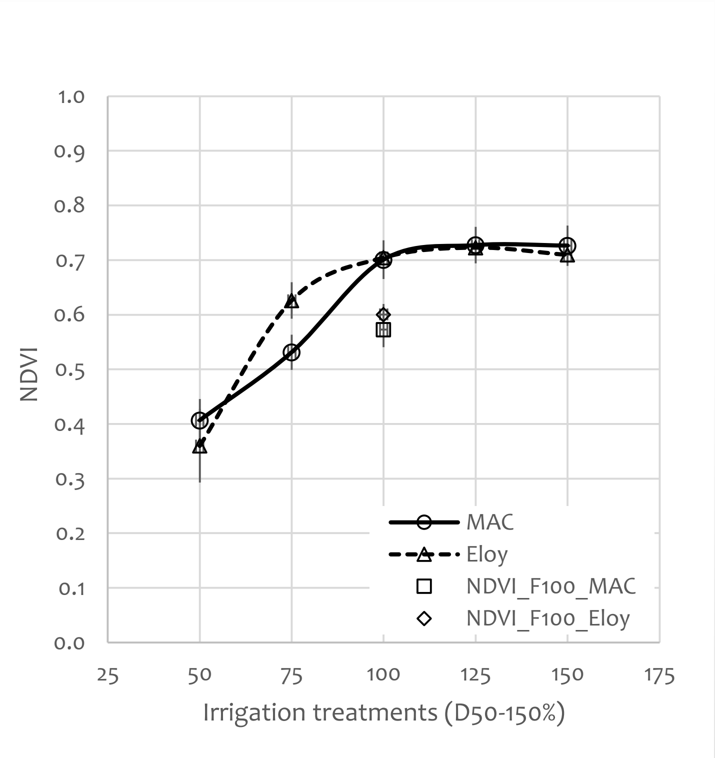
Normalized vegetation index, biomass, rubber and resin yield at MAC and Eloy

Site	Treatment	Dry biomass Kg/ha	Rubber yield Kg/ha	Resin yield Kg/ha
MAC	D50	11787	358	809
	D75	15075	421	941
	D100	14674	393	904
	D125	14262	383	868
	D150	14814	396	933
	F100	12947	411	853
Eloy	D50	14235	383	879
	D75	13359	310	796
	D100	14455	314	817
	D125	13547	280	741
	D150	13105	268	720
	F100	15775	367	878



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IR camera and TDR (soil) sensors

See poster presentation by Danielle Murdoch-Hoare and Matt Katterman.

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WINDS model

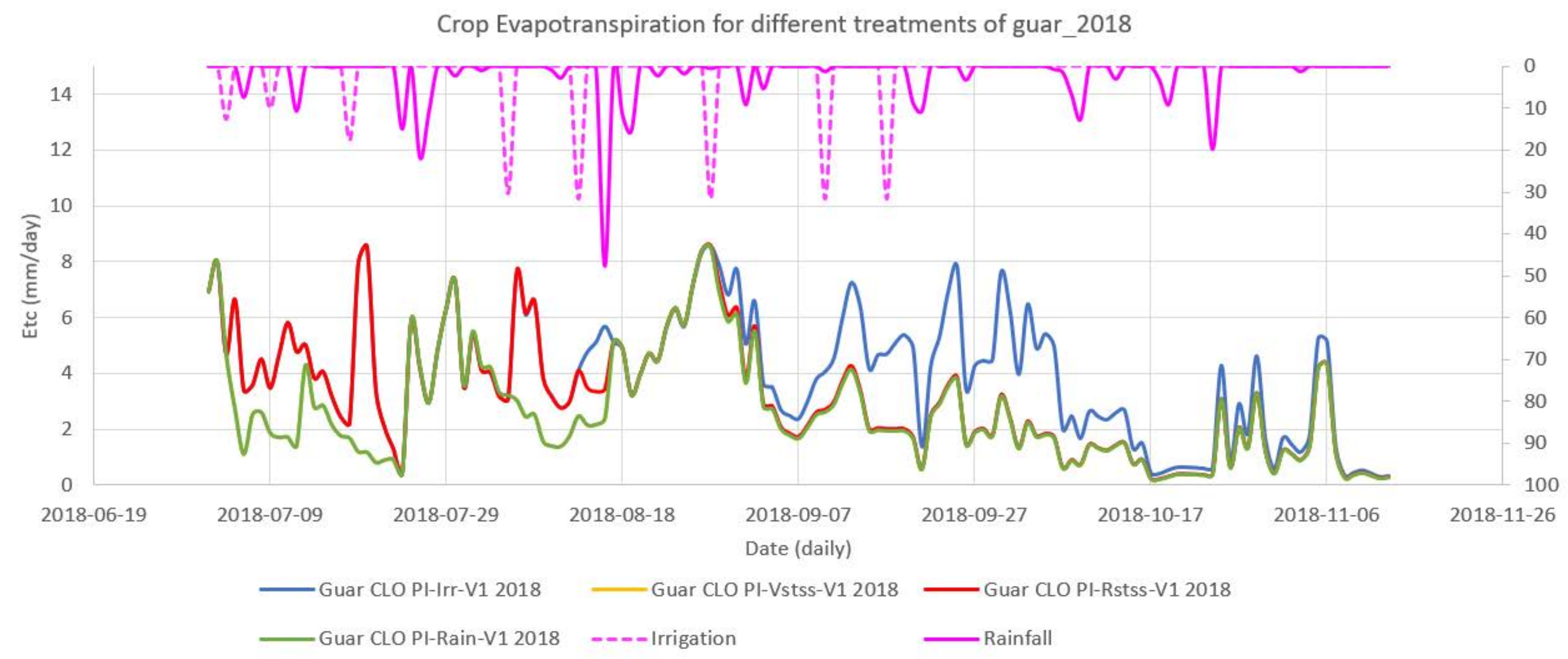
MAC (guayule) and Clovis (guar)

All data in database on server (high value for future analysis)

Data accessible with FLASK python program (website data access)

Developing website and phone app.

AAIC conference PowerPoints available upon request.



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Tasks for year 3

- Guayule irrigation
  - ***Harvest*** *the guayule plants* ***2 years*** *after planting (in April 2020) at MAC and Eloy.* ***Re-grow*** *the guayule plants at both sites, and* ***developing*** *the basal crop coefficient curve for the third year. Continue all remote and in-situ measurements*
  - *Begin* ***new flood irrigation*** *scheduling experiment at Eloy, installation of sensors and assist with management of irrigation and remote sensing.*
  - ***Continue*** *WINDS model development and analysis*
  - *Use* ***sensors*** *to detect crop status and stress*
- Guar irrigation
  - ***Continue*** *WINDS model development and analysis*
  - *Continuing to partner with Sangu Angadi on irrigation analysis in Clovis*

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Deficit irrigation experiment

- The experiment will be a split plot design with the variety as the main plot and the sub-plots (irrigation treatment) will be arranged in a randomized complete block design (distributed randomly in each of the 3 Reps).
- **The irrigation treatments are:**
  1. ***Full irrigation (control treatment): Irrigate as determined by the model.***
  2. ***Half Irrigation: Irrigate every other irrigation as determined by the model.***
  3. ***Minimum irrigation years 1&2: Irrigate three times a year, approximately every growth stage (May/June, September, and February/March).***
  4. ***Minimum Year2: Year 1 irrigate as determined by the model, and Year 2 irrigate three times (February, May/June, and September).***
  5. ***One irrigation: one irrigation after establishment in the first year, one irrigation in year 2.***

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Managing Weeds in Guayule Year 2 Accomplishments

- 23 PREE experiments in 3 locations
- Six herbicides: acetochlor, bensulide, ethalfluralin, metolachlor, pendimethalin, sulfentrazone
- Three incorporation methods: sprinkler, PPI on the flat, PPI on bed-top.
- Three planting/irrigation methods in course textured soils (i.e., sandy soils)

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Managing Weeds in Guayule Year 2 Accomplishments

- 14 Postemergence herbicide studies
- 12 Carfentrazone (Aim) broadleaf herbicide studies
  - *Topical sprays on 4 growth stages: 2, 4, 6, and 9 leaf guayule seedlings*
  - *Also included paraquat in 2 experiments*
- 2 Studies with grass herbicides: clethodim, fluazifop-p-butyl, and sethoxydim

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Managing Weeds in Guayule Year 2 Accomplishments

- Extensive discussions with scientists at BASF, Bayer, Corteva, FMC, Gowan, and Syngenta
  - *24c SNL labels*
- Conducted tour of Bridgestone Research Farm in Eloy, field plots at the Maricopa Ag. Center and Bridgestone processing plant in Mesa.

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Managing Weeds in Guayule Year 3 Goals

- In Maricopa and Eloy, conduct Fall 2019 and Spring 2020 guayule herbicide tolerance studies
- Continue PREE studies and POST broadleaf herbicide studies
- Post-directed herbicides studies
- Herbicide application sequences for chemical weed control from seeding to 6 month old plants.
- Submit data to chemical companies and ADA for 24c SLN labels

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Evaluating Guayule Germplasm for Salt Tolerance

- **EC=Electrical Conductivity (decisiemens/m). Higher values=more salt. Can have detrimental to adverse effect on crops. Effects vary by variety.**

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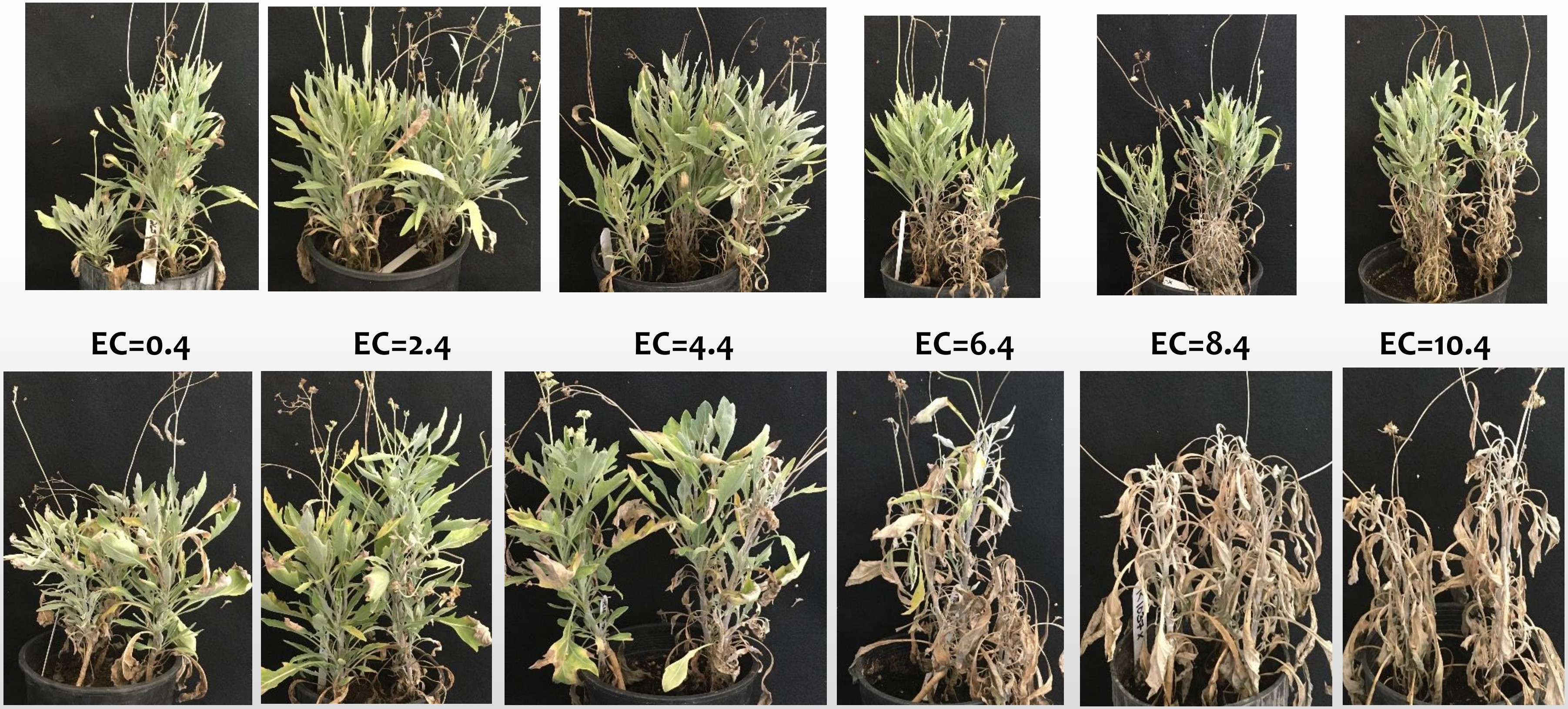
Germplasm Line 4265F (Top)
Germplasm Line R1037X (Bottom)



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Root Structure Between Planting Methods

Transplant

Direct-seed

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49 DAP

	Plant height	Aove Ground Biom.	Root 1 (0 - 20 cm)	Root 2 (20 - 40 cm)	Root 3 (40 - 60 cm)	Root 4 (60 - 80 cm)	Total Mean Root Wt (g)
	Mean (cm)	Mean (g)	Mean	Mean	Mean	Mean	
Direct-seed	9.38a	0.52	0.09	0.03	0.03	0.01	0.16
Trans-plant	15.5b*	2.70*	0.63*	0.22*	0.21	0.1	1.16*

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109 DAP

	Plant height	Above Ground Biom.	Root 1 (0 - 20 cm)	Root 2 20 - 40 cm)	Root 3 (40 - 60 cm)	Root 4 (60 - 80 cm)	Root 5 (80 - 100 cm)	Root 6 (100 - 120 cm)	Total Mean Root Wt (g)
	Mean (cm)	Mean (g)	Mean (g)	Mean (g)	Mean (g)	Mean (g)	Mean (g)	Mean (g)	Mean (g)
Direct-seed	22.9	11.0	2.9	1.1	0.7	0.8	0.6	0.0	6.1
Trans-plant	22.4	14.6	4.8	2.1	1.9	1.9	1.5	0.6	12.8

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Feedstock Accomplishments (Bridgestone)

Plant Density Study – 1 year data

- AZ-2 is a lot more vigorous as individual plants and populations
- Sensor measurements captured plant growth adequately
- When canopy is closed, correlations between biomass and NDVI were not great.
- Rubber and resin content in one year old plants differ between AZ-2 and Sel-1, but doesn't seem to respond to plant population.

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Feedstock Development and Production

- Objective 5: Develop soil quality and health knowledge critical to environmental sustainability.

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Accomplishments and PIs

- MAC and Eloy irrigation field trials sampled in (54 *samples/field/year*)
- Year 1 samples analyzed for soil texture, soil chemistry, and soil microbial community
- Plant samples collected for analysis

- Diaa El-Shikha (U of A)
- Colleen McMahan (USDA)
- Julia Neilson (U of A)
- Peter Waller (U of A)

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Sustainable Feedstock Production
Soil Health and Guayule Productivity

Julie Neilson, Raina Maier, Kyle Brown, Lia Ossanna

- **Project 1** **Profile soil health during guayule irrigation field trial**

Objective:

Quantify soil health data associated with irrigation treatment, guayule biomass, and guayule rubber and resin production.

Results:

- MAC and Eloy irrigation field trials sampled in Year 1 (5/2017) and Year 2 (3/2018; *54 samples/field/year*)
- Year 1 samples analyzed for **soil texture** (*clay content*), **soil chemistry** (*pH, EC, OrgM, NO_3-N, P, K*), and **soil microbial community** (*soil DNA extraction*).
- Plant samples collected for analysis; March 2018

Significance to SBAR Project goals:

- **Soil quality metrics potentially associated with guayule productivity: *% clay, phosphorus concentration, pathogen abundance, mycorrhizal fungi***

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Project 2: Guayule-soil microbiome interactions during winter dormancy

Objective:

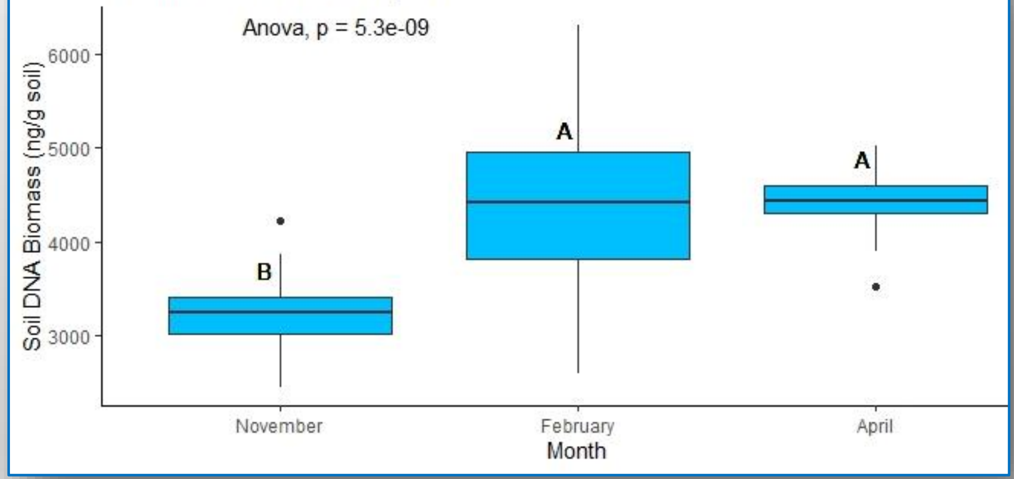
Evaluate associations between guayule winter dormancy, rubber production, and microbial community dynamics (*Drip100 MAC field treatment; winter 2019*).

Sampling times and associated guayule growth stage:

November 2018	peak rubber transferase production
February 2019	peak rubber production
April 2019	end of winter dormancy

Root zone soil

Soil DNA biomass



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Significance of Soil Health Research to SBAR Project

- Identify critical soil health dynamics that impact guayule growth and rubber production and can inform field management practices
- Identify associations between microbial community dynamics and growth-stage associated rubber production metrics to expand our mechanistic understanding of factors driving the initiation of rubber production during winter dormancy.

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FEEDSTOCK DEVELOPMENT & PRODUCTION
USDA-ARS Rubber Lab (McMahan Lab)
Key Tasks for Year 3

- *Sub Obj: 2) Downregulate flowering to improve yield - guayule*
  - Finalize 2 publications on gene expression of flowering/non-flowering plants.
  - Continue additional transformations (LEAFY) until Dec 2019.
  - Recover plants and evaluate genotypes (DNA, RNA) and phenotypes (rubber content, plant architecture, etc.) through Year 3.

- *Obj. 5. Develop soil quality and health knowledge critical to environmental sustainability.*
  - **Complete data analysis of physiological and chemical characteristics of samples collected during Year 2 winter dormancy growth study with Julia Neilson.**

    Plant architecture, carbon fixation rate, gene expression, rubber biosynthesis rate, rubber particle and molecule characteristics

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Sustainable Feedstock Production
Soil Health and Guayule Productivity
Year 3 Key Tasks

- **Soil health profile**: MAC and Eloy irrigation field trials

  Biogeochemical analysis of Year 2 samples; Year 3 sample collection immediately after harvest in March 2020.

- **Temporal microbial community analysis** of samples collected during Year 2 winter dormancy growth study with Colleen McMahan.

  Identify associations between microbial community dynamics and specific rubber production metrics

- **Identify dynamic changes** in complex bacterial/archaeal/fungal community interactions that associate with different guayule growth stages.

  Network analysis of the guayule root zone microbiome of samples collected from August 2019 through April 2020; Eloy irrigation field trial

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POST-HARVEST LOGISTICS & CO-PRODUCTS

LEAD: *Catie Brewer*

Team: *Armijo, Audu, Bayat, Carillo-Little, Cheng, DeDecker, Dehghanizadeh, Fan, Gill, Gunatilaka, Gutierrez, Holguin, Jarvis, Jena, Laje, Madasu, Montoya, Mozaffari, Muraviyov, Polisetti, Pradyawong, Rosalez, Rodriguez-Uribe, Sehar, Soliz, Usrey, White, Wright, Zuniga-Vazquez*

Post-Harvest Logistics & Co-Products Year 2 Status Update

- Fan Group (Transportation/Optimization Modeling, presented with Sustainability)
- Gunatilaka Group (Co-Product Chemistry and Activity)
- Holguin Group (Biomass and Co-Product Chemistry)
- Brewer & Jena Groups (Bagasse Conversion and Resin Separation)

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Isolation & Identification of Major & Biologically-Active Co-Products in Guayule (& Guar) (Gunatilaka, Molnar, Xu, Chandrashekar & Liu @ UA)

12 New Compounds (%) ← **26 Compounds** → **14 Known Compounds (%)**

1. **SiO2 Column chromatography**
2. **C-18 RP HPLC**

- **Argentatin A (0.018), B (0.70, C (0.60), D (0.06)**
- **Carissone (0.064)**
- **Guayulin D (0.023)**
- **g-Eudesmol (0.003)**
- **3-*Epi*-argentatin D (0.160)**
- ***Iso*-argentatin A (0.005)**
- ***Iso*-argentatin B (0.062)**
- **Linoleic acid (0.013)**
- **Quisquagenin (0.013)**
- **3*b*-Hydroxy *iso*-argentatin A (0.002)**
- **3*b*-Hydroxy argentatin C (0.012)**

Purified Argentatin B (1.0 g), Argentatin C (200 mg), and a mixture of Argentatin A and *Iso*-argentatin A (2.0 g) were provided for studies at Bridgestone

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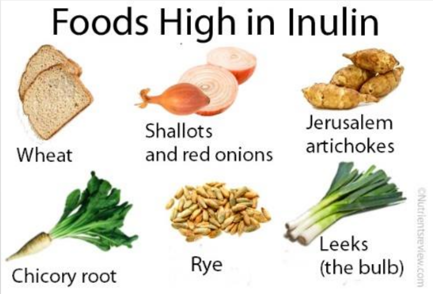
Isolation & Quantification of Inulin from Guayule Bagasse

- **Inulin: fructans; polysaccharides of fructose with glucose chain ends.**
- **Inulin is known to have health benefits and food applications:**
  - ***Inulin is used in food as a fat and sugar replacer, and as a dietary (soluble) fiber and prebiotic***
  - ***Inulin can prevent gastrointestinal complications like constipation***
  - ***Inulin gels used as drug delivery vehicles for water soluble-drugs and as stabilizing matrices for labile drugs***
  - ***Inulin consumption enhances mineral absorption of minerals and stimulates the immune system***
- **Global market for Inulin: >250,000 tons in 2015**

Expected 480,000 tons and >$2.5 billion by 2023

(https://www.grandviewresearch.com/industry-analysis/inulin-market)



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Isolation & Quantification of Inulin from Guayule Bagasse

Bagasse (Bridgestone ID, 2017-7-1, Bay-233)
(500.0 g)

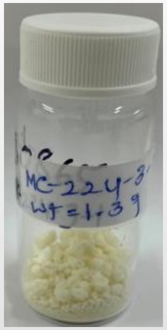
1. **Soxhlet extraction with MeOH (3.0 L)**
2. **Extraction of Residue with Bioling water (3 x 2.2 L)**

H_2O extract
(wt = 46.5 g)   (12.5 g)

1. **Dissolved in water (50 mL)**
2. **Added EtOH (950 mL)**

Crude Polysaccharide
(10.1 g)

1. **Deprotenization using $CHCl_3$: n-BuOH (4:1)**
2. **Removed of polyphenols by polyclar column**
3. **Decolorization using H_2O_2 - H_2O (1:10)**



Inulin powder
(1.3 g; ca. 1.0%
from bagasse)

Inulin quantified as fructose in the crude polysaccharide hydrolysate:

HPLC method: ~13.3%

Spectrophotometric method: ~23.5%

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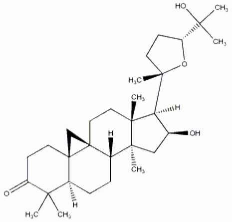
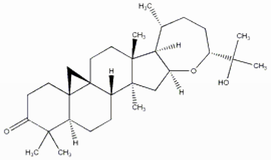
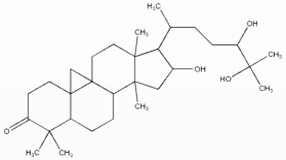
Guayule Value-Added Co-Products – Year 3







- Chemical & microbial transformations of argentatins A, B, and C
- Evaluate transformation products of argentatins A, B, and C for anticancer and antimicrobial activities
- Isolate and characterize major metabolites of guayule terpene solution; attempt chemical and microbial transformations to produce co-products with anticancer and antimicrobial activities

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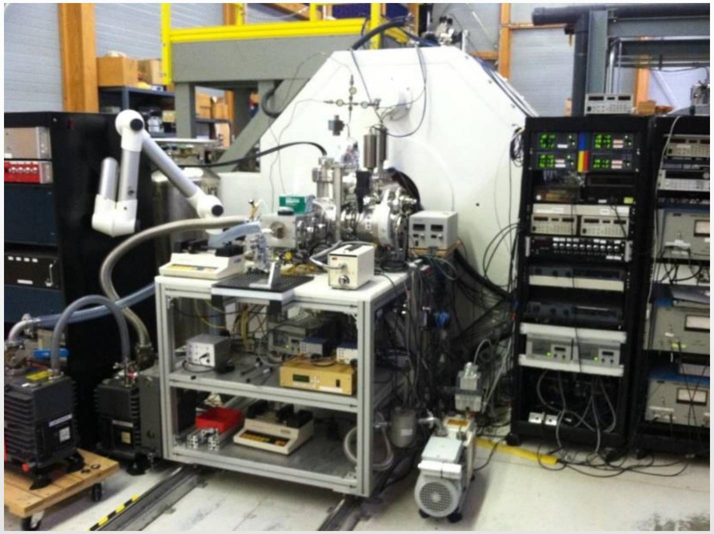
Chemical Analysis
(Holguin group @ NMSU)

- Completed first draft of the guayule cold tolerance manuscript
- Completed extension article: Natural Products in the Southwest: Guayule and Guar
- Performed high-resolution mass spectroscopy of guayule resin
- Profiled triacyl glycerides and wax esters in guayule leaves and resin
- Measured galactomannan content and size in guar from 2017 and 2018
- Isolated *Rhizobium* from nodules of guar plants grown at NMSU Leyendecker Farm



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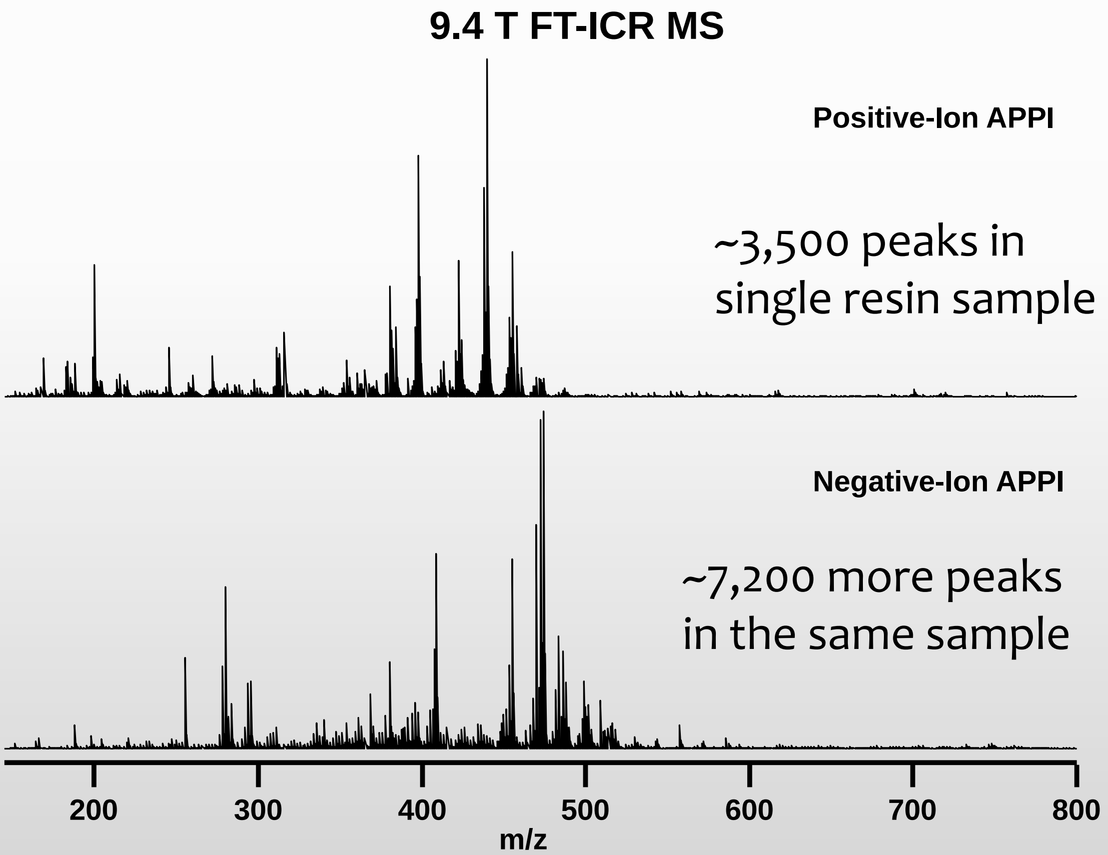
Ultrahigh Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectroscopy



~3,500 peaks in single resin sample

~7,200 more peaks in the same sample

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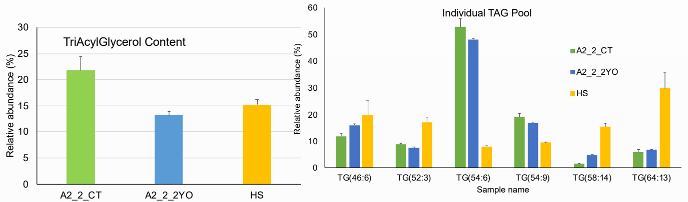
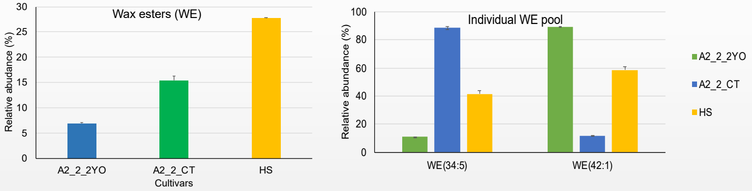
Guayule Metabolomics and Lipidomics

- Cold acclimation first draft "Metabolic Responses of Guayule (*Parthenium argentatum* A. Gray) to Cold Acclimation and Freezing"
- Triacyl glycerides (TAGs) and wax esters (Wes) content in guayule leaves
- Putatively identified 7 abundant TAGs in resin

TAGs



WEs



Triacylglycerol Profiling of Resin

Input Mass	Matched Mass	Delta	Name	Formula
890.8398	890.8171	0.0227	TG(53:2)	$C_{56}H_{104}O_{6}$
892.9034	892.8328	0.0706	TG(53:1)	$C_{56}H_{106}O_{6}$
894.9207	894.8484	0.0723	TG(53:0)	$C_{56}H_{108}O_{6}$
870.9246	870.7545	0.1701	TG(52:5)	$C_{55}H_{96}O_{6}$
896.9158	896.7702	0.1456	TG(54:6)	$C_{57}H_{98}O_{6}$
872.9048	872.7702	0.1346	TG(52:4)	$C_{55}H_{98}O_{6}$
874.984	874.7858	0.1982	TG(52:3)	$C_{55}H_{100}O_{6}$

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Guar Highlights

- Galactomann isolation, purification, and size estimation by aqueous size exclusion chromatography
- Galactomann enzymatic yield from guar seed endosperm samples
- Isolation of *Rhizobium* from nodules of guar plants

Nitrogen fixing guar nodules

Rhizobium isolated from nitrogen fixing Guar nodules

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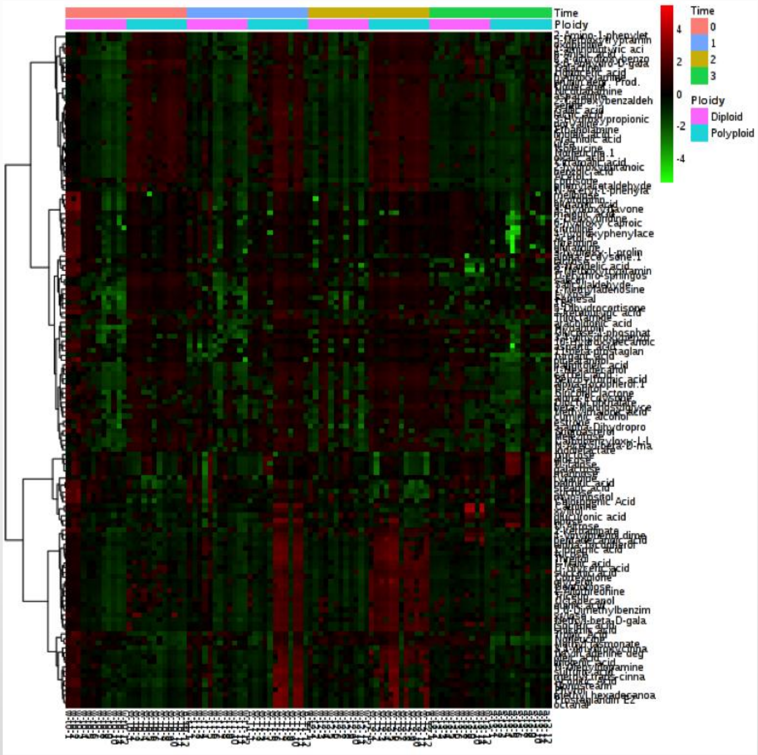
Guar and Guayule Chemical Analysis – Year 3

- Biochemical composition analysis of guar, guayule, and co-products
- Metabolomic biomarker identification for improved performance of guar and guayule varieties
- Analytical evaluation of guayule bagasse thermochemical conversion products

Metabolomic Profile of Guayule



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Bagasse Conversion & Resin Separation (Brewer & Jena groups @ NMSU)

- Hydrothermal liquefaction (HTL) of guayule bagasse

- Improving energy recovery for wastewater treatment co-processing low moisture content (MC) bagasse and high MC algae

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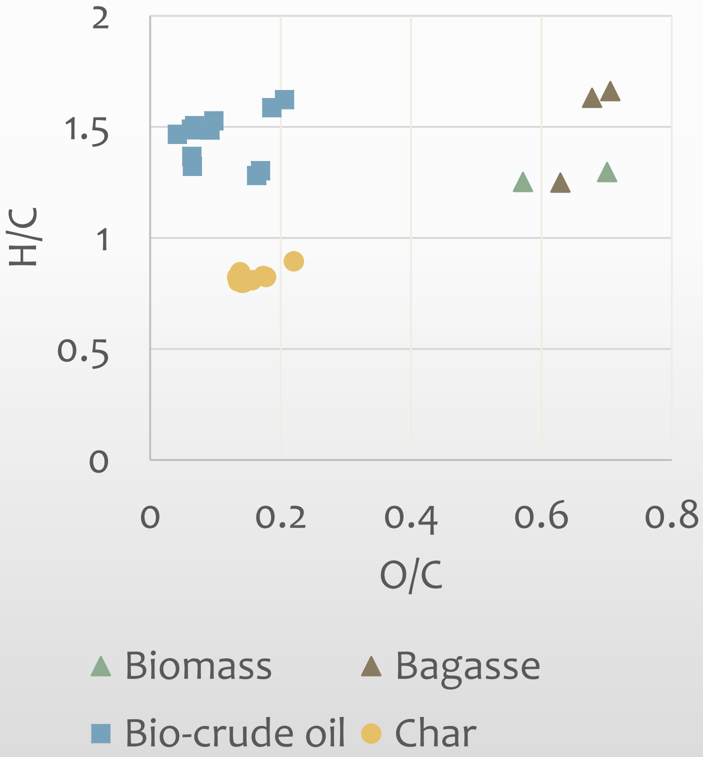
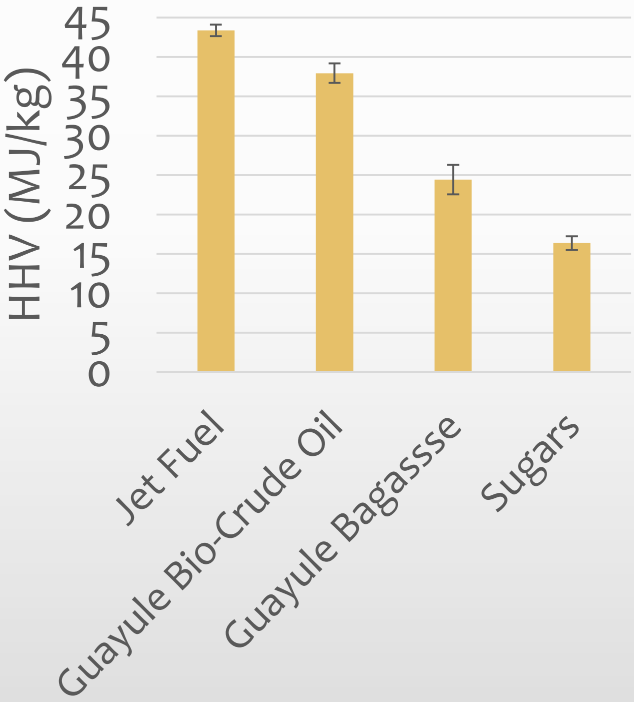
Guayule Bagasse Conversion

Comparison of elemental and energy content between biomass and conversion products: CHNSO and higher heating value (HHV)





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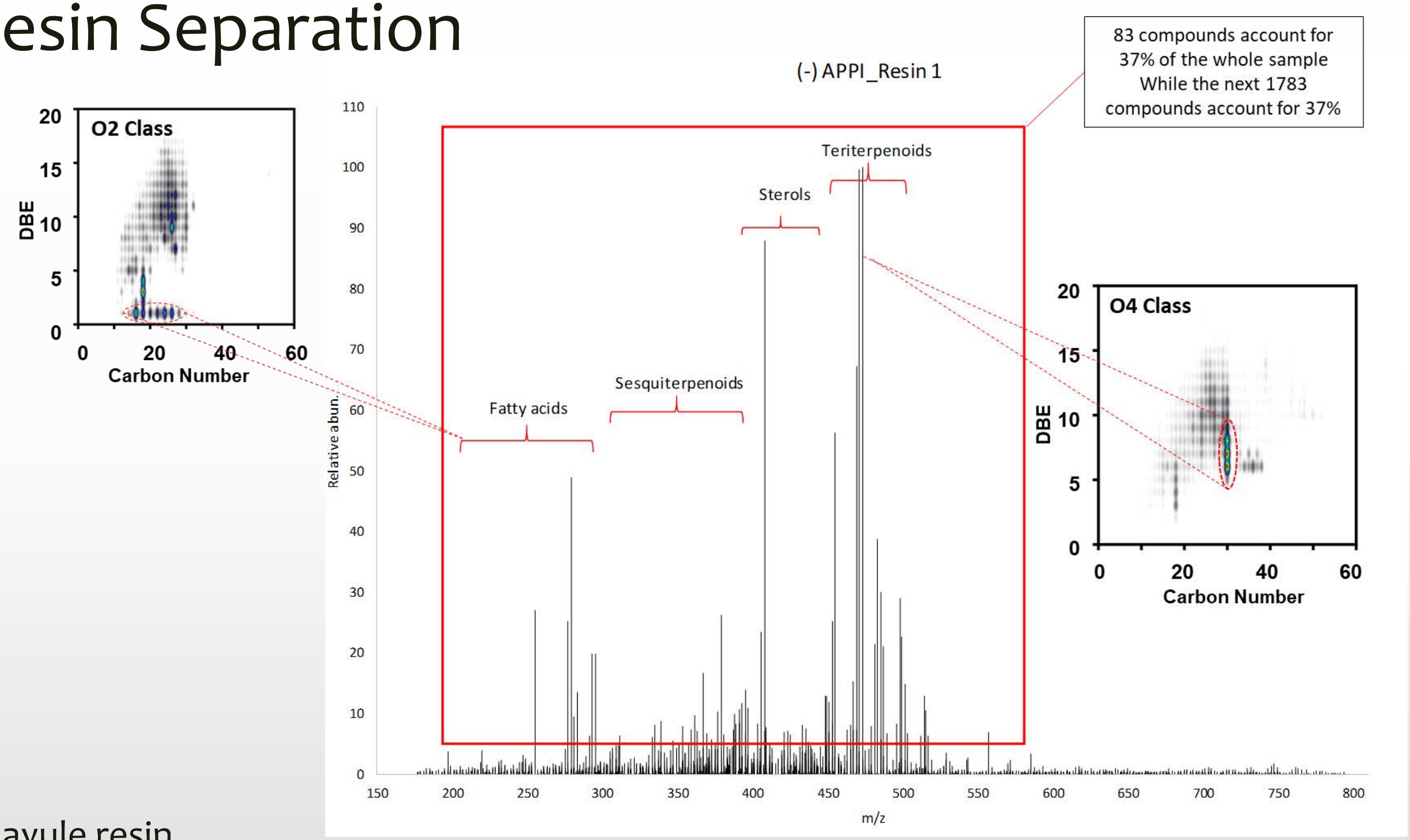
Resin Separation



83 compounds account for 37% of the whole sample While the next 1783 compounds account for 37%

Guayule resin

- Gas chromatography for terpenes
- Liquid chromatography
- New insights: high-resolution Fourier transform ion cyclotron resonance mass spectroscopy

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Bagasse Conversion & Resin Separation – Year 3

- Bagasse characterization support for field studies
- Co-HTL of algae and bagasse
- Guayule resin fractionation
  - *Supercritical CO2*
  - *Liquid-liquid*
  - *Green solvents*
  - *Filtration*

ASE 350® Accelerated Solvent Extractor Operator's Manual. Document No. 065220. Revision 01. April 2008

Adopted from Waters® MV10 ASFE™ system https://www.waters.com

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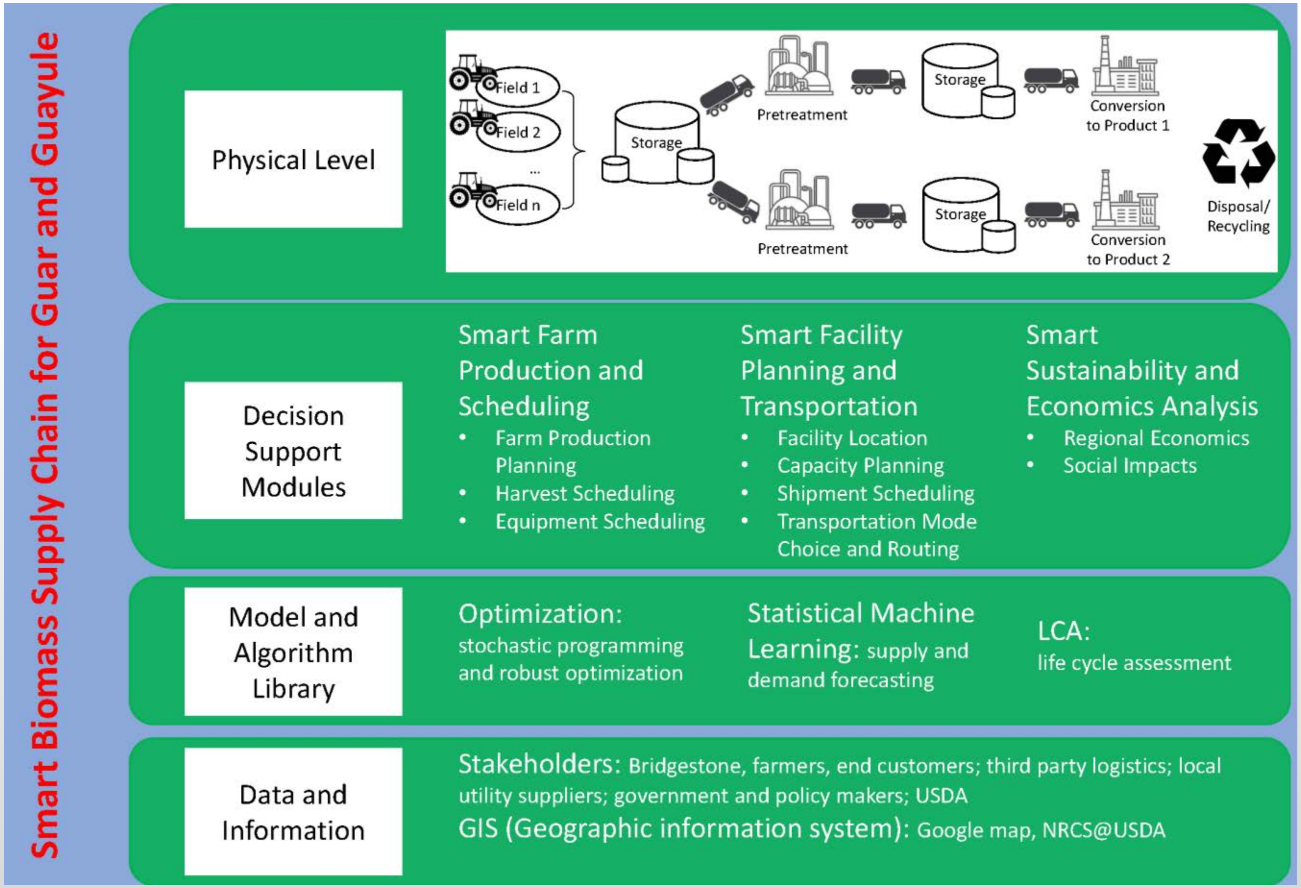
Model and Algorithm Development for Biomass Supply Chain (Fan Group @ UA)



Smart Biomass Supply Chain for Guar and Guayule

Physical Level

Decision Support Modules

Smart Farm Production and Scheduling
- Farm Production Planning
- Harvest Scheduling
- Equipment Scheduling

Smart Facility Planning and Transportation
- Facility Location
- Capacity Planning
- Shipment Scheduling
- Transportation Mode Choice and Routing

Smart Sustainability and Economics Analysis
- Regional Economics
- Social Impacts

Model and Algorithm Library

Optimization: stochastic programming and robust optimization

Statistical Machine Learning: supply and demand forecasting

LCA: life cycle assessment

Data and Information

Stakeholders: Bridgestone, farmers, end customers; third party logistics; local utility suppliers; government and policy makers; USDA

GIS (Geographic information system): Google map, NRCS@USDA

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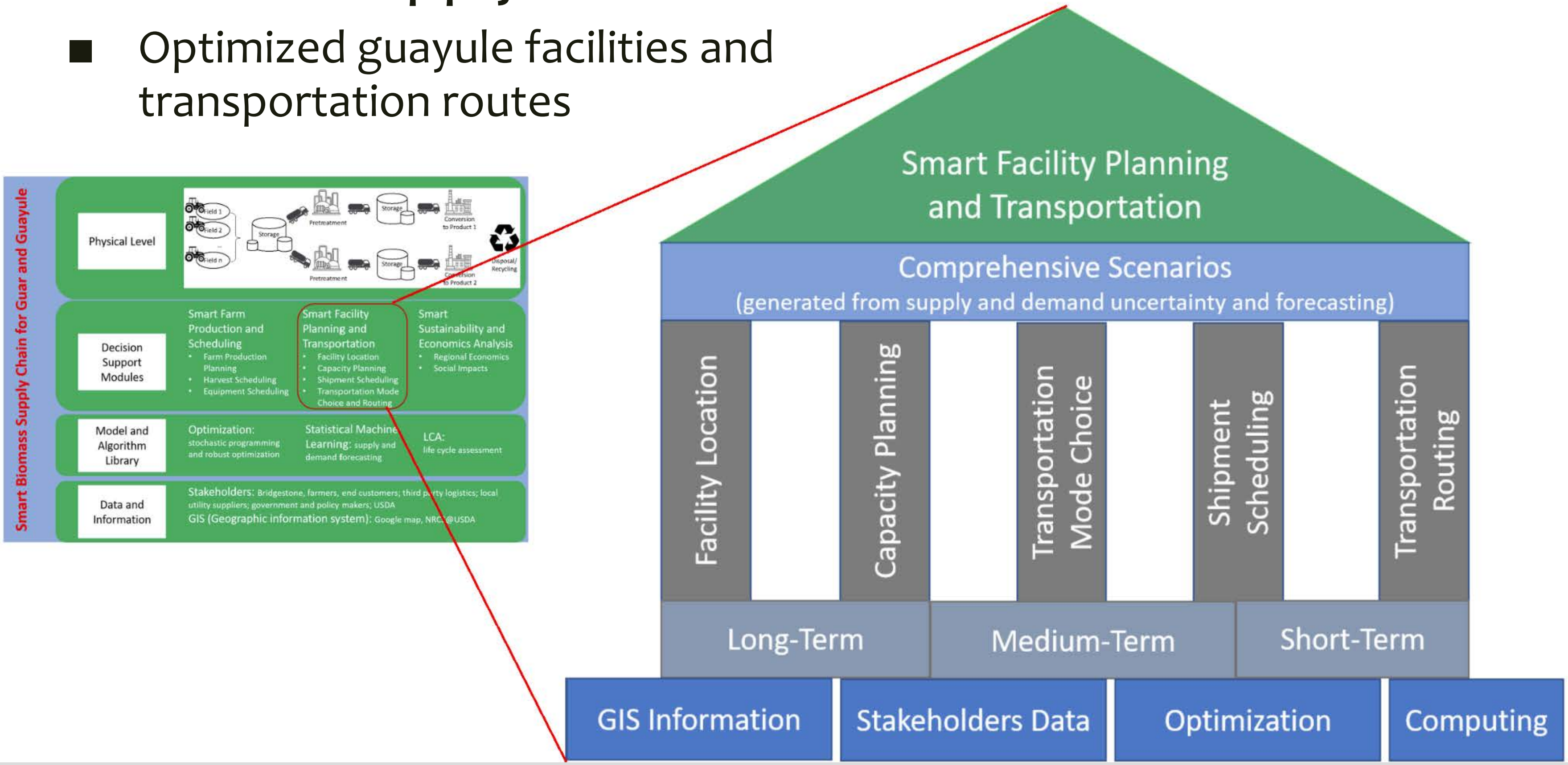
Model and Algorithm Development for Biomass Supply Chain

- Optimized guayule facilities and transportation routes



O. Sun, N. Fan, GIS-based two-stage stochastic facility location problem considering planting plan uncertainty, *submitted for publication, 8/2019.*

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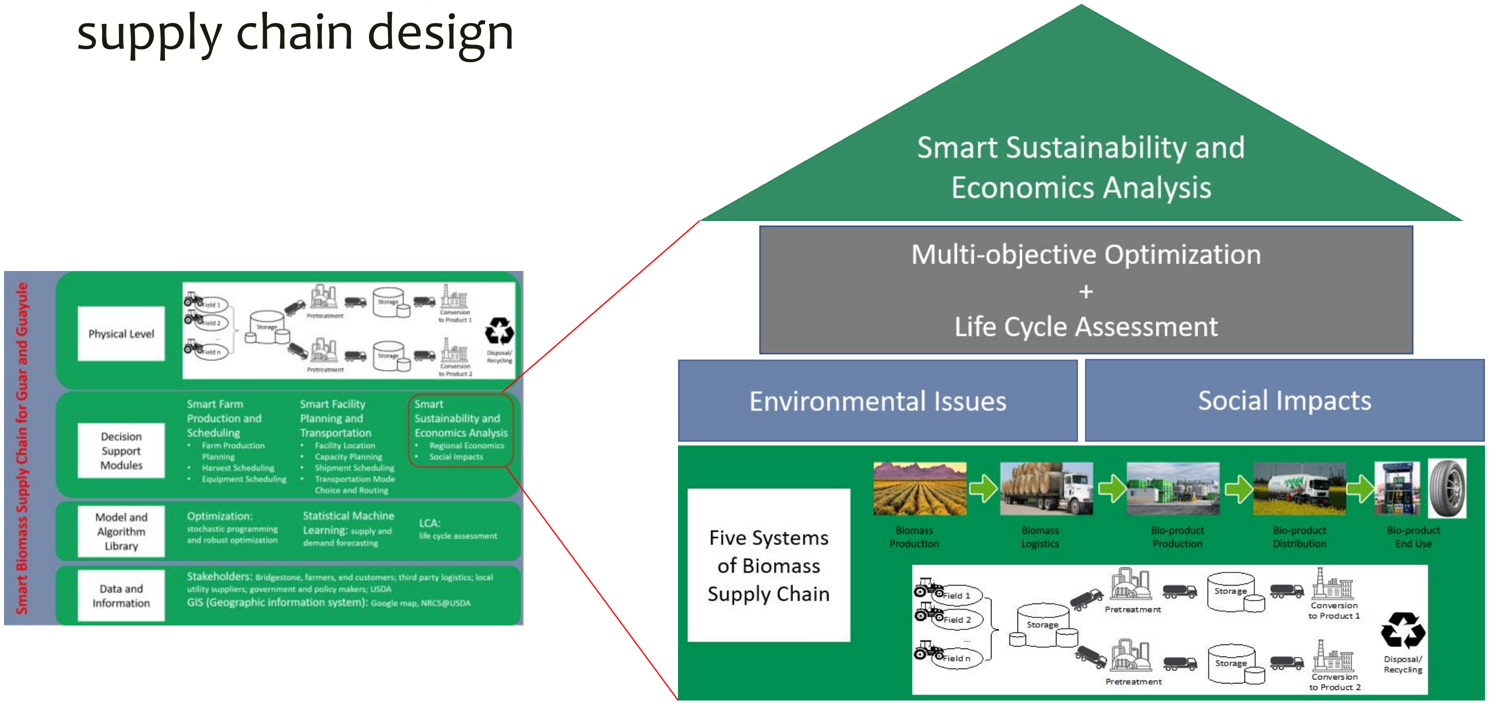
Model and Algorithm Development for Biomass Supply Chain

- Integrating sustainability and economics analysis into biomass supply chain design



Smart Biomass Supply Chain for Guar and Guayule

Physical Level

Decision Support Modules

- Smart Farm Production and Scheduling
  - Farm Production Planning
  - Harvest Scheduling
  - Equipment Scheduling
- Smart Facility Planning and Transportation
  - Facility Location
  - Capacity Planning
  - Shipment Scheduling
  - Transportation Mode Choice and Routing
- Smart Sustainability and Economics Analysis
  - Regional Economics
  - Social Impacts

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Optimization: stochastic programming and robust optimization

Statistical Machine Learning: supply and demand forecasting

LCA: life cycle assessment

Data and Information

Stakeholders: Bridgestone, farmers, end customers; third party logistics; local utility suppliers; government and policy makers; USDA

GIS (Geographic information system): Google map, NRCS@USDA

Smart Sustainability and Economics Analysis

Multi-objective Optimization
+
Life Cycle Assessment

Environmental Issues

Social Impacts

Five Systems of Biomass Supply Chain

✓ *D.A. Zuniga Vazquez, graduate poster presentation, 9/2019.*

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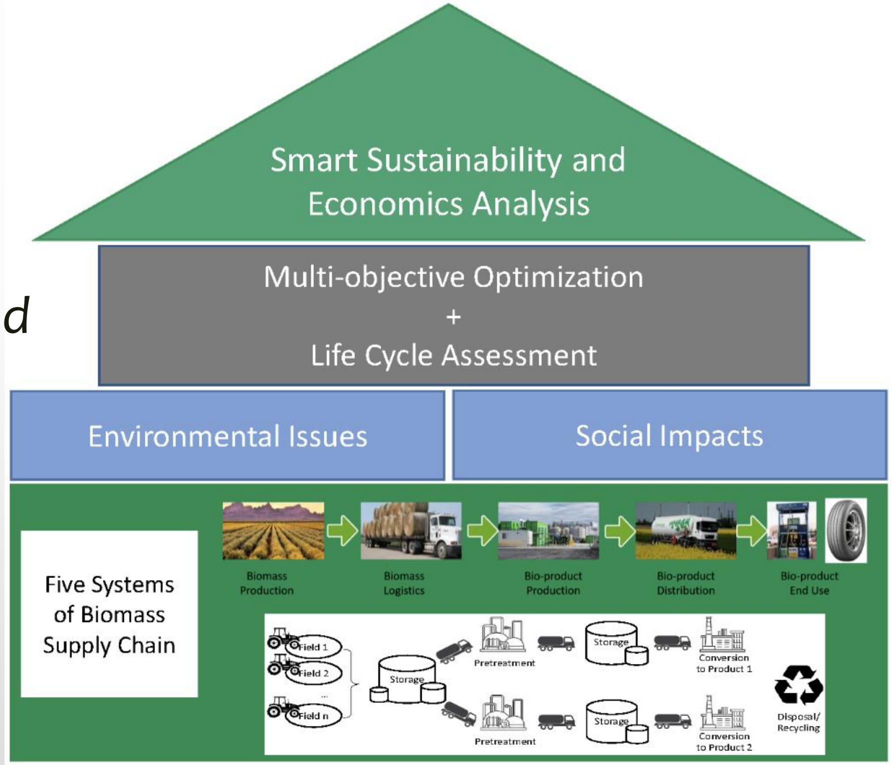
Model and Algorithm Development for Biomass Supply Chain – Year 3

- Comprehensive sustainability and regional economics analysis based on most current data from industrial partners, literature and sustainability group
    - *minimize total annual capital and operation cost*
    - *minimize total annual CO2-equivalent GHG emission*
    - *maximize accrued local jobs*
- Apply integer optimization approaches to design smart farm production plan and scheduling



Smart Sustainability and Economics Analysis

Multi-objective Optimization
+
Life Cycle Assessment

Environmental Issues

Social Impacts

Five Systems of Biomass Supply Chain

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SYSTEM PERFORMANCE & SUSTAINABILITY

LEAD: *Jason Quinn*

Team: *Acharya, Bhandari, Brewer, Fan, Gutierrez, Khanal, Landis, Mealing, Ogden, Rogstad, Teegerstrom, Seavert, Sproul, Summers, White, Zuniga-Vazquez*

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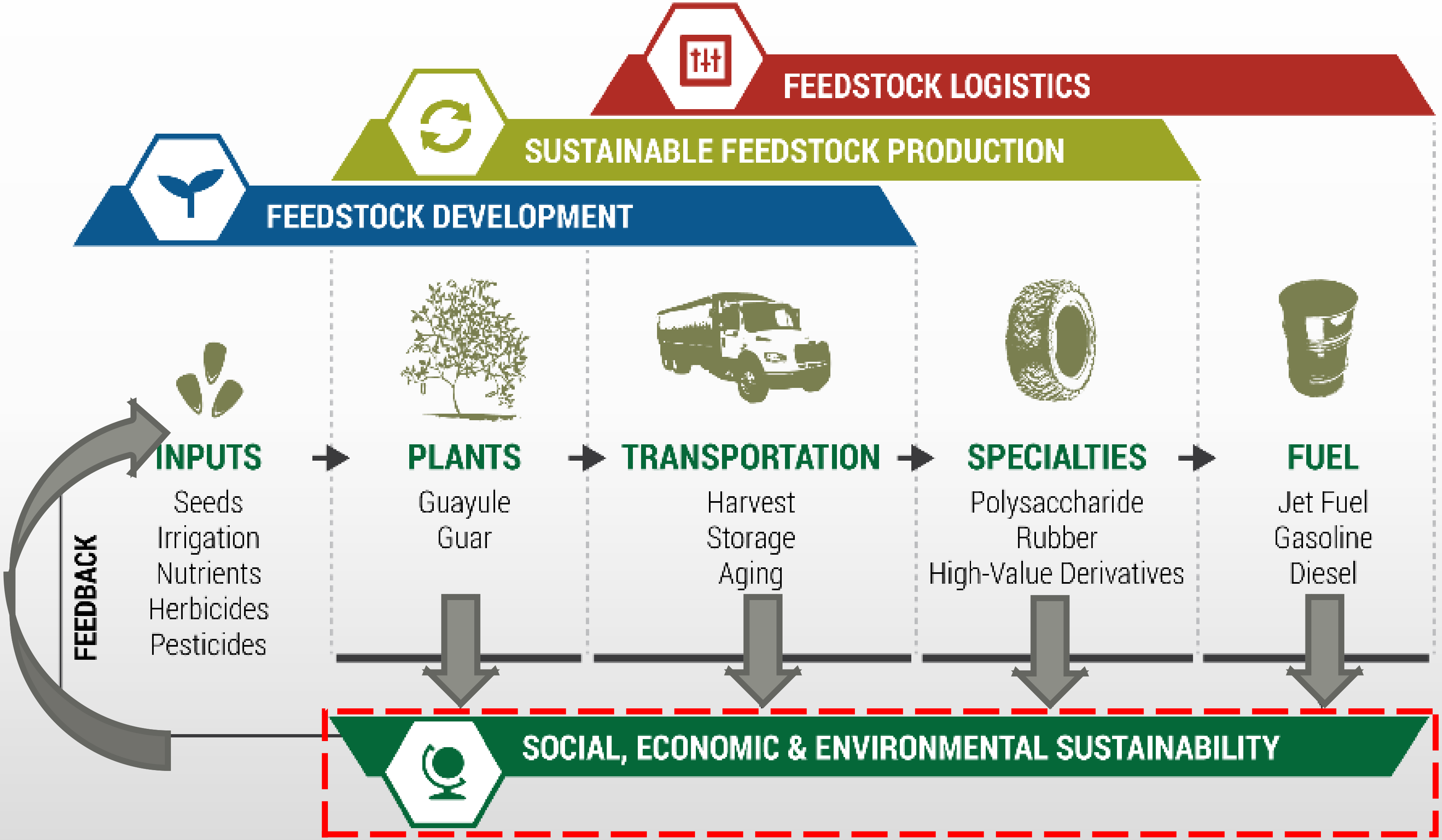

FEEDSTOCK LOGISTICS

SUSTAINABLE FEEDSTOCK PRODUCTION

FEEDSTOCK DEVELOPMENT

INPUTS → **PLANTS** → **TRANSPORTATION** → **SPECIALTIES** → **FUEL**

INPUTS	PLANTS	TRANSPORTATION	SPECIALTIES	FUEL
Seeds Irrigation Nutrients Herbicides Pesticides	Guayule Guar	Harvest Storage Aging	Polysaccharide Rubber High-Value Derivatives	Jet Fuel Gasoline Diesel

FEEDBACK

SOCIAL, ECONOMIC & ENVIRONMENTAL SUSTAINABILITY

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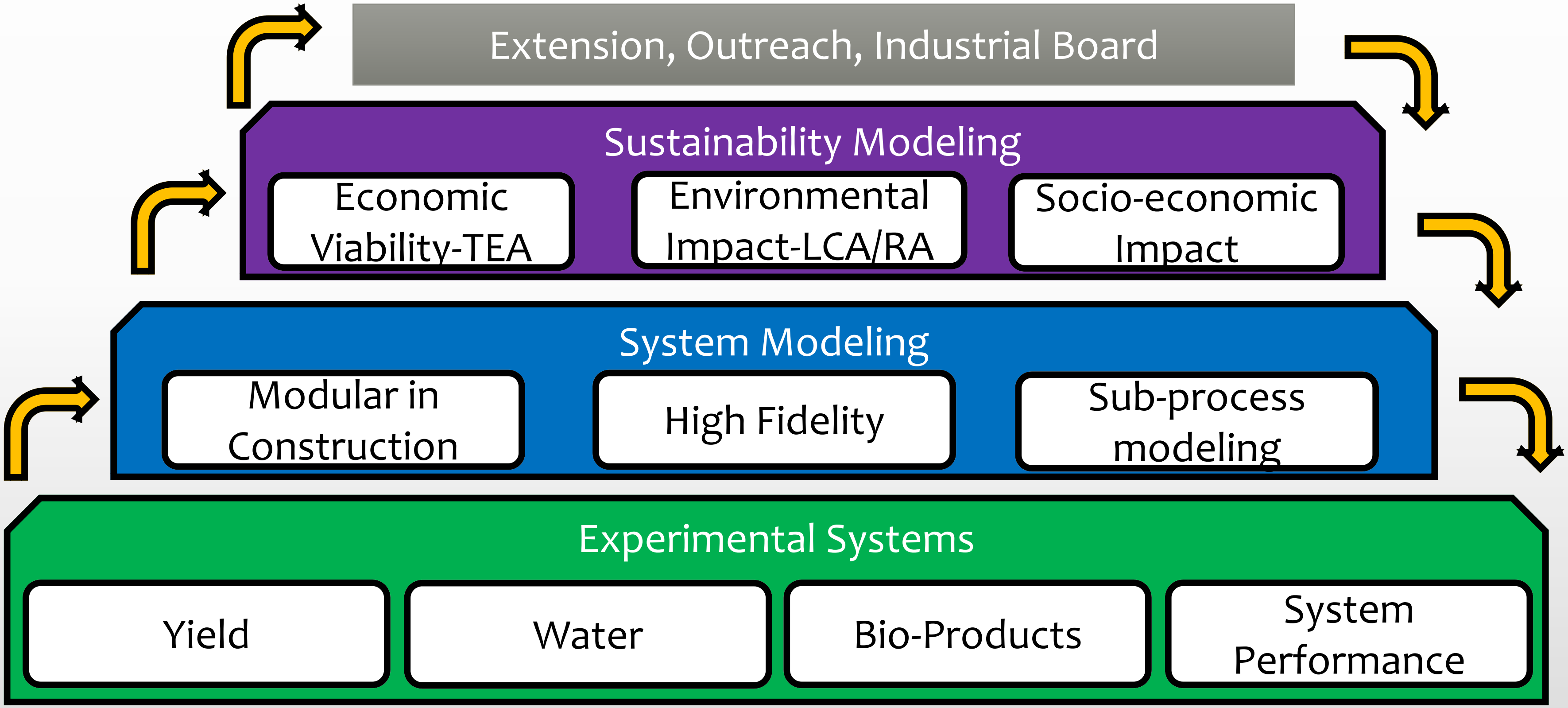
Data Flow



Extension, Outreach, Industrial Board

Sustainability Modeling

Economic Viability-TEA

Environmental Impact-LCA/RA

Socio-economic Impact

System Modeling

Modular in Construction

High Fidelity

Sub-process modeling

Experimental Systems

Yield

Water

Bio-Products

System Performance

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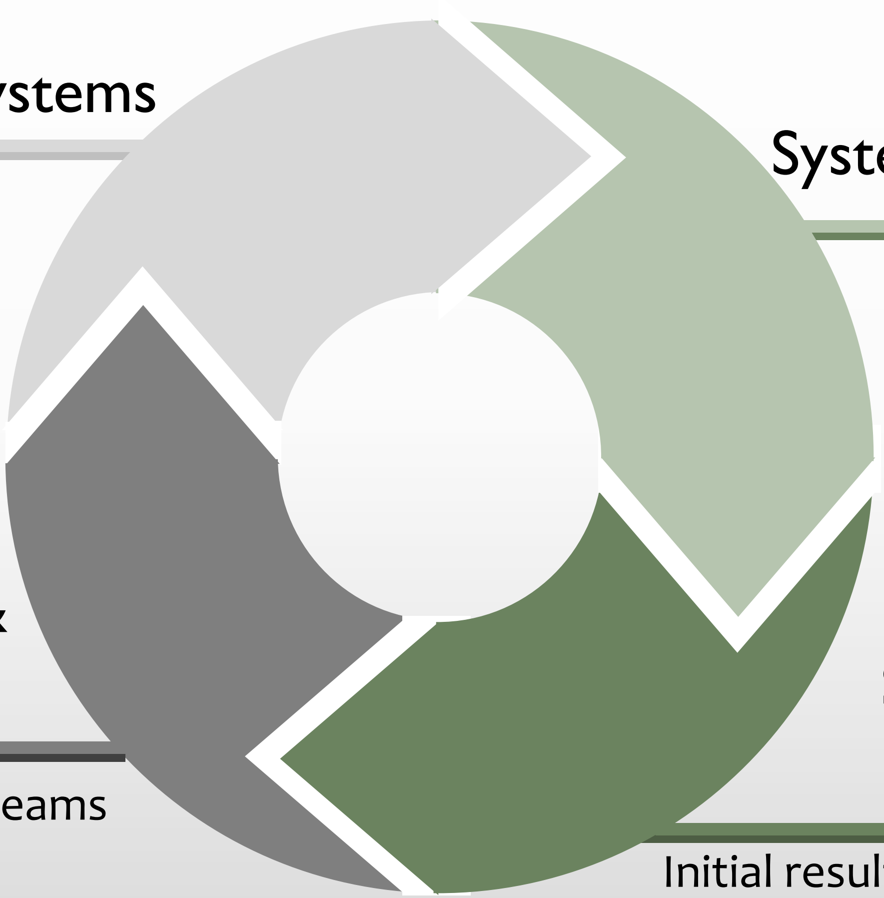
Sustainability Team



Experimental Systems

Identified high-impact targets

System Modeling

Integration complete: *Energy and Mass balances*

Sustainability Modeling

Initial results

- Costs/benefits of production
- Environmental Impacts

Data Feedback & Validation

Interfacing across teams

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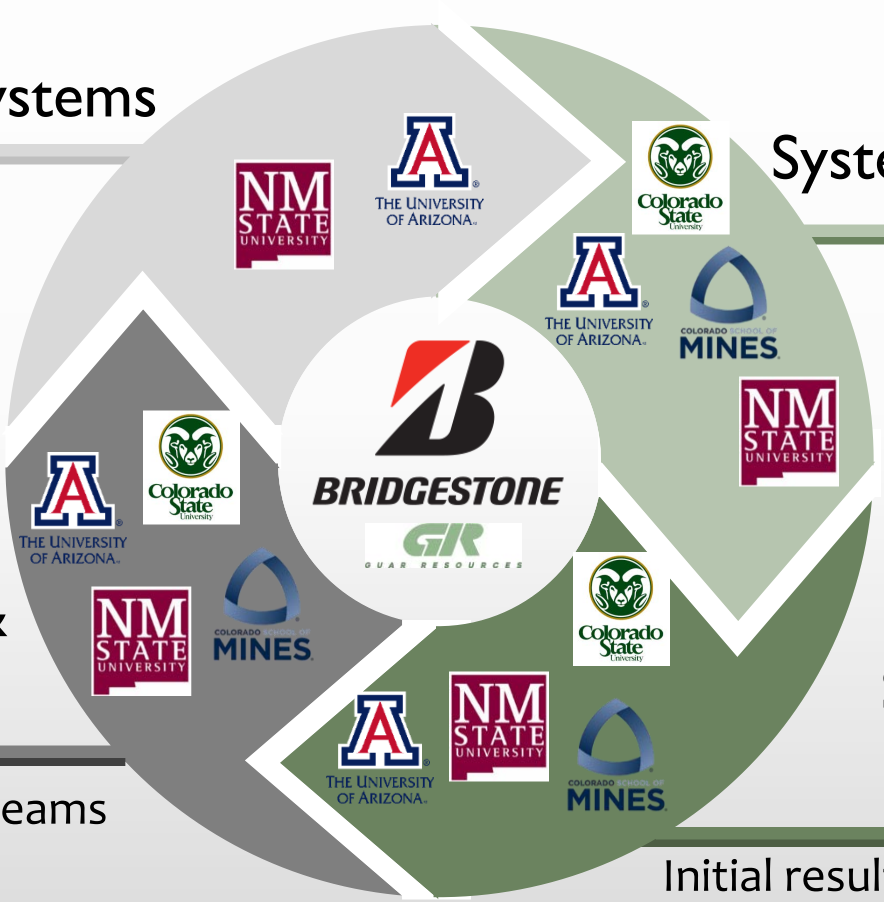
Sustainability Team



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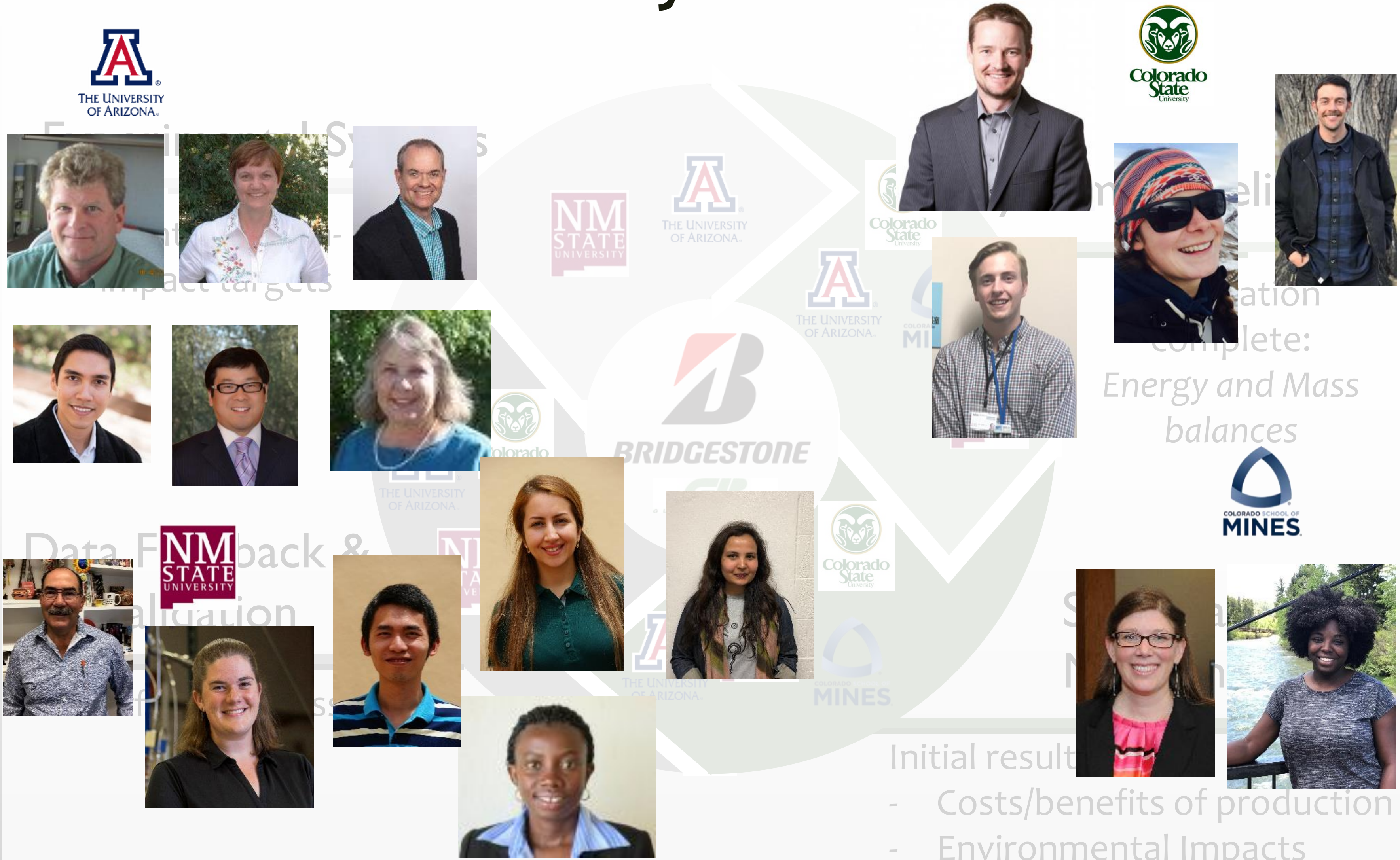

Energy and Mass balances

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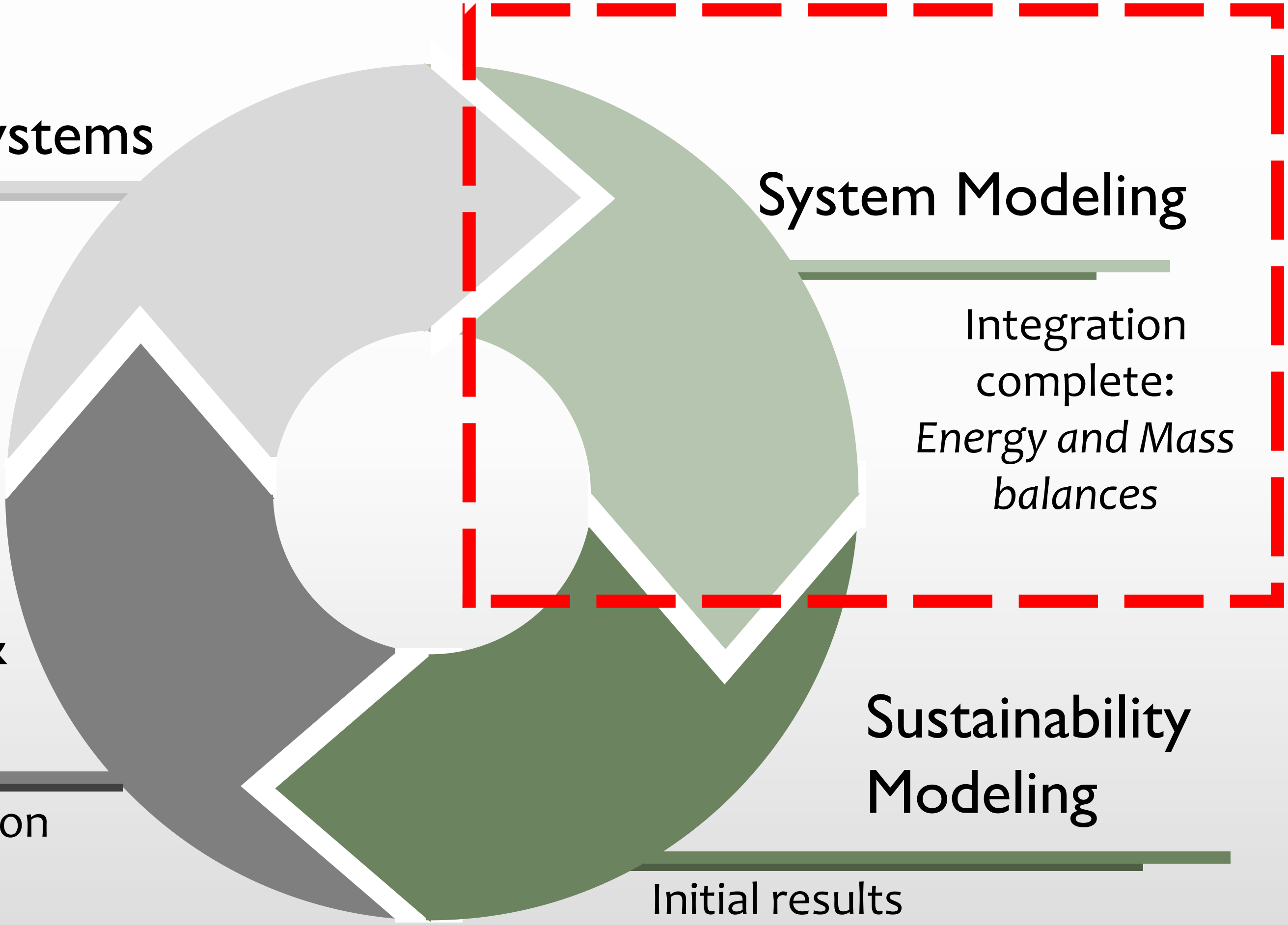

Experimental Systems

Identified high-impact targets

System Modeling

Integration complete: *Energy and Mass balances*

Data Feedback & Validation

De-risking production

Sustainability Modeling

Initial results
- Costs/benefits of production
- Environmental Impacts

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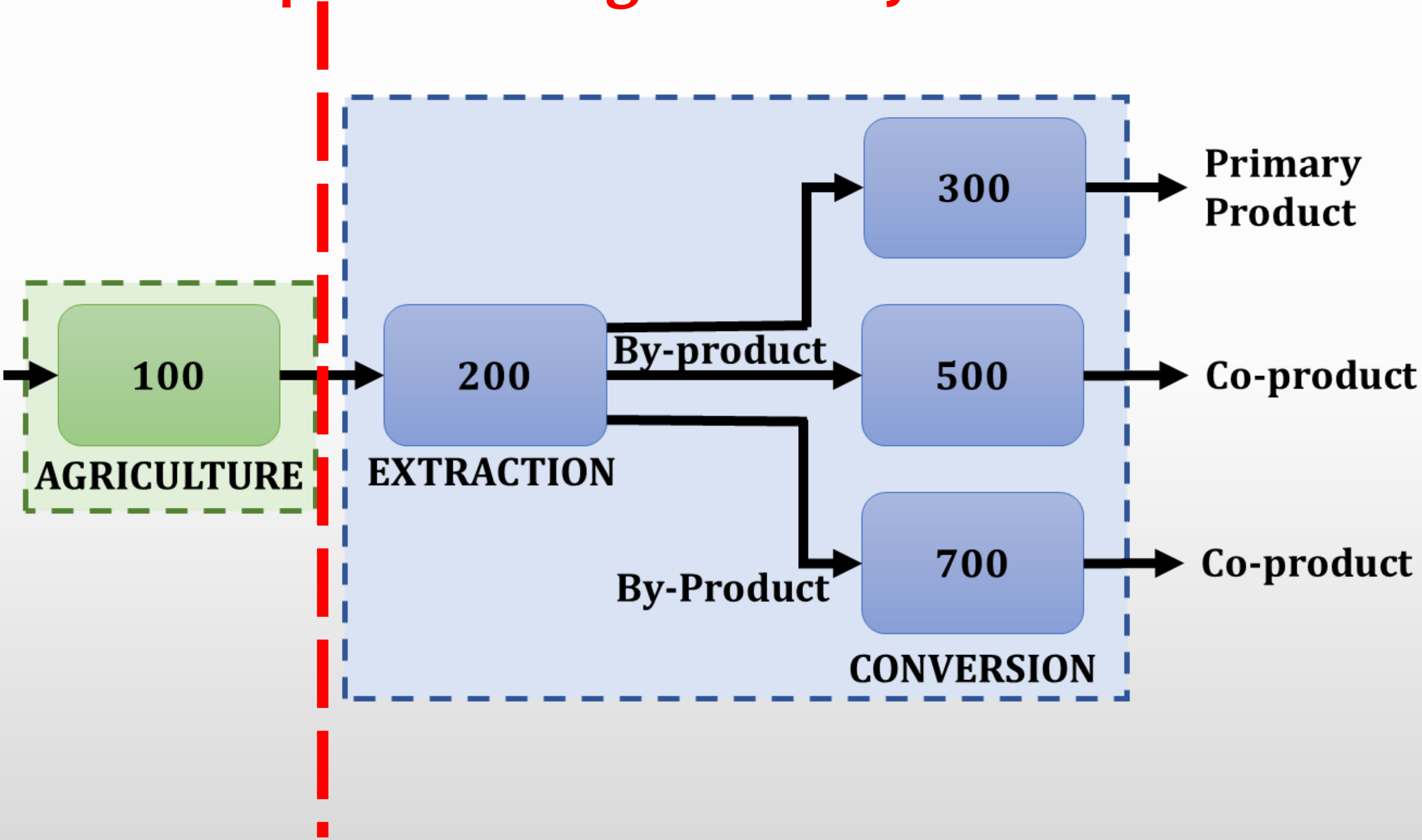
System Modeling

Disconnect prior to integration in year 2



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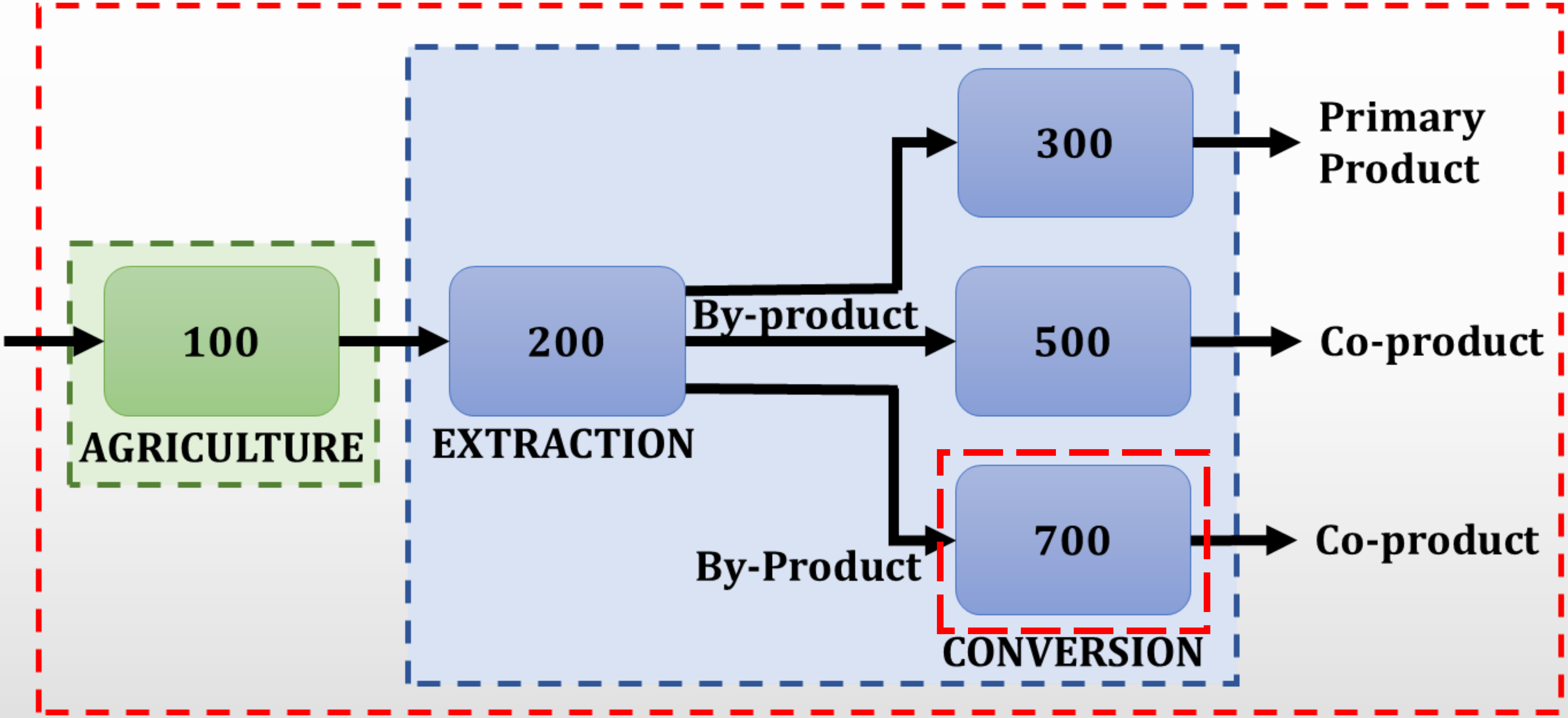
System Modeling



Fully Integrated energy and mass balance cross the entire value chain

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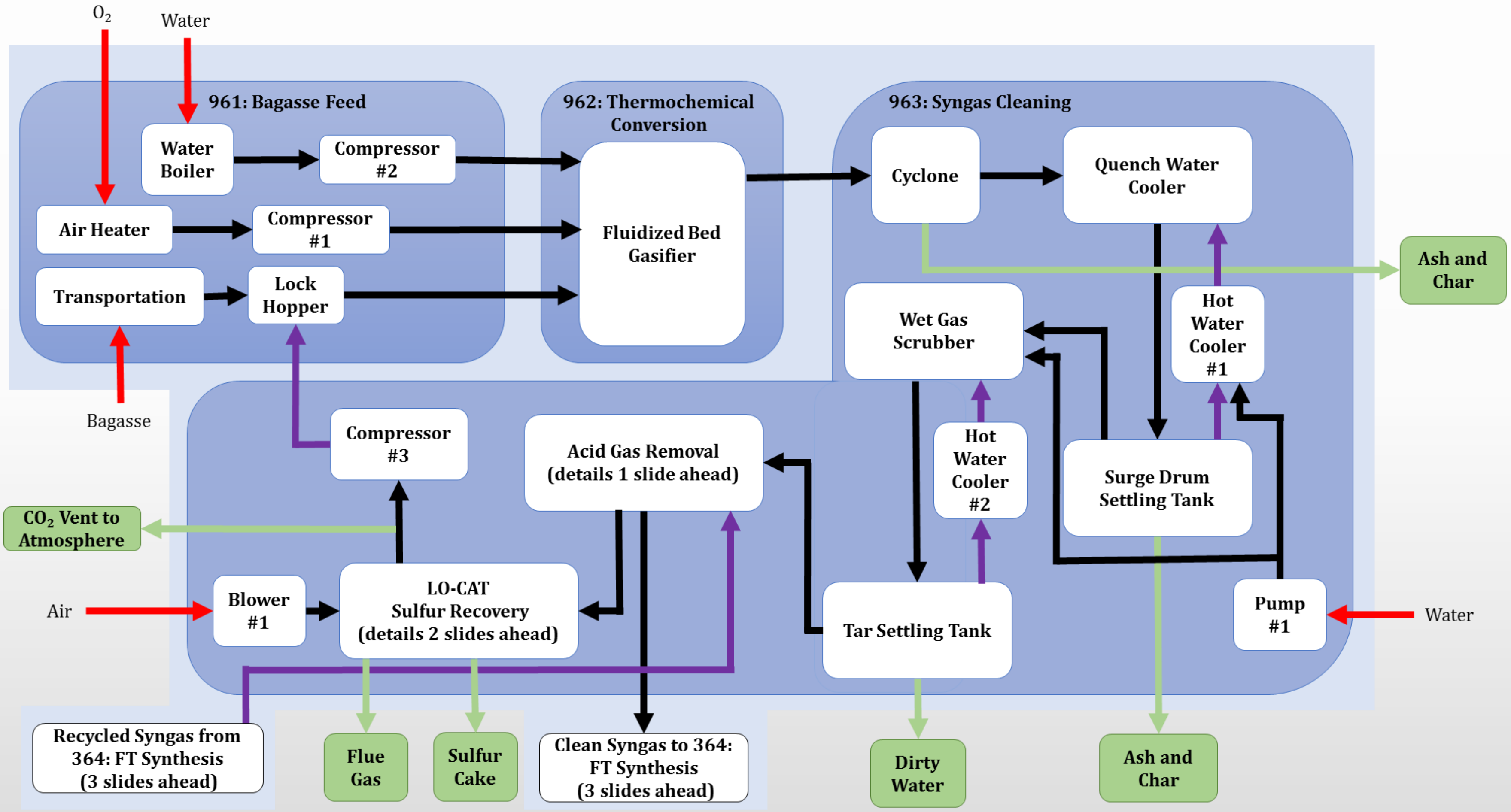
System Modeling:

Bagasse Conversion Process via Gasification


O_2

Water

961: Bagasse Feed

Water Boiler

Compressor #2

Air Heater

Compressor #1

Transportation

Lock Hopper

Bagasse

962: Thermochemical Conversion

Fluidized Bed Gasifier

963: Syngas Cleaning

Cyclone

Quench Water Cooler

Ash and Char

Wet Gas Scrubber

Hot Water Cooler #1

Hot Water Cooler #2

Surge Drum Settling Tank

Pump #1

Water

Tar Settling Tank

Compressor #3

Acid Gas Removal (details 1 slide ahead)

CO_2 Vent to Atmosphere

Air

Blower #1

LO-CAT Sulfur Recovery (details 2 slides ahead)

Recycled Syngas from 364: FT Synthesis (3 slides ahead)

Flue Gas

Sulfur Cake

Clean Syngas to 364: FT Synthesis (3 slides ahead)

Dirty Water

Ash and Char

Complete energy and mass balance at unit operation level

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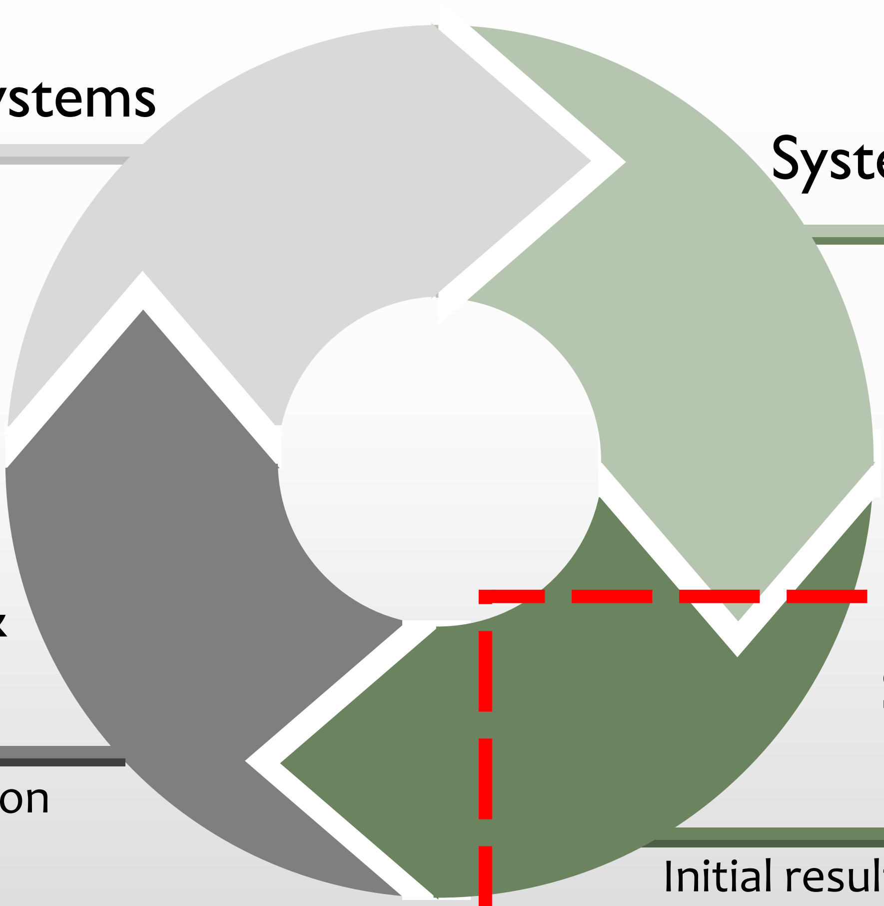
Sustainability Team



Experimental Systems

Identified high-impact targets

System Modeling

Integration complete:
Energy and Mass balances

Data Feedback & Validation

De-risking production

Sustainability Modeling

Initial results

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TEA & LCA Methods : Guayule

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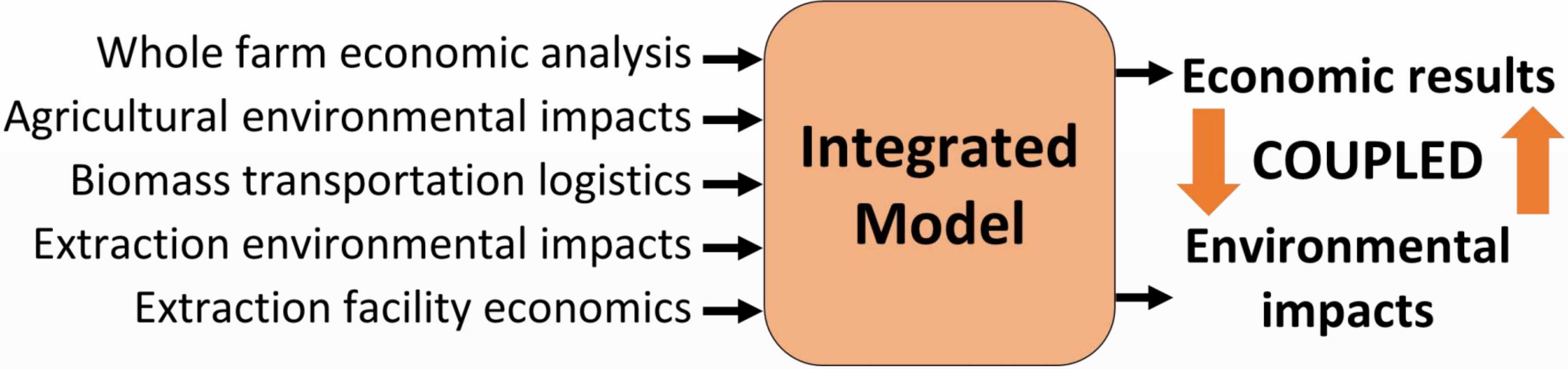

Whole farm economic analysis →
Agricultural environmental impacts →
Biomass transportation logistics →
Extraction environmental impacts →
Extraction facility economics →

Integrated Model

→ **Economic results**

COUPLED

→ **Environmental impacts**

Discounted Cash Flow Rate of Return

Environmental impact: TRACI 2.1

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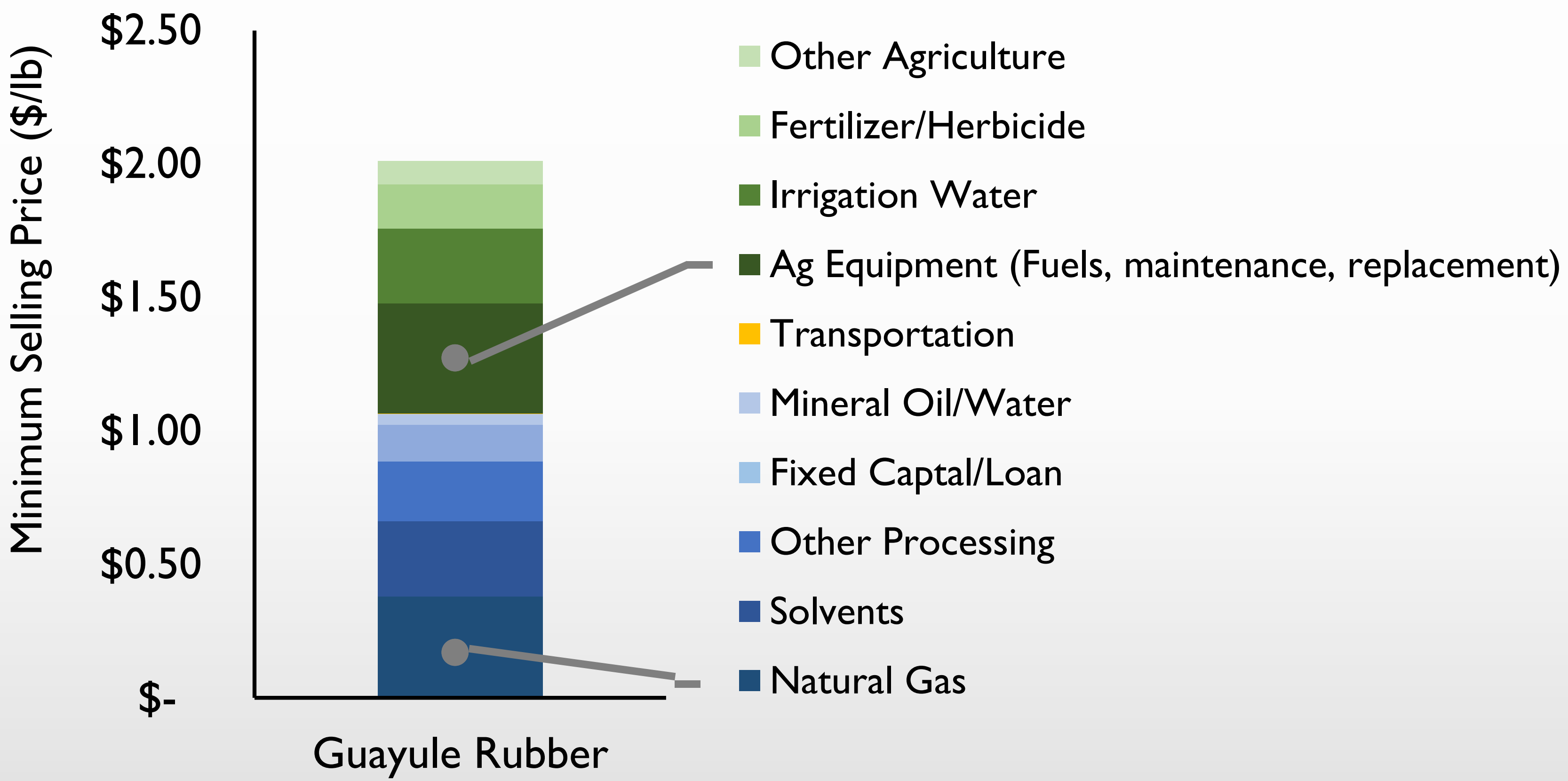
Baseline Integrated Economic Results - Guayule



Robust Baseline Results: Highlighted impact areas

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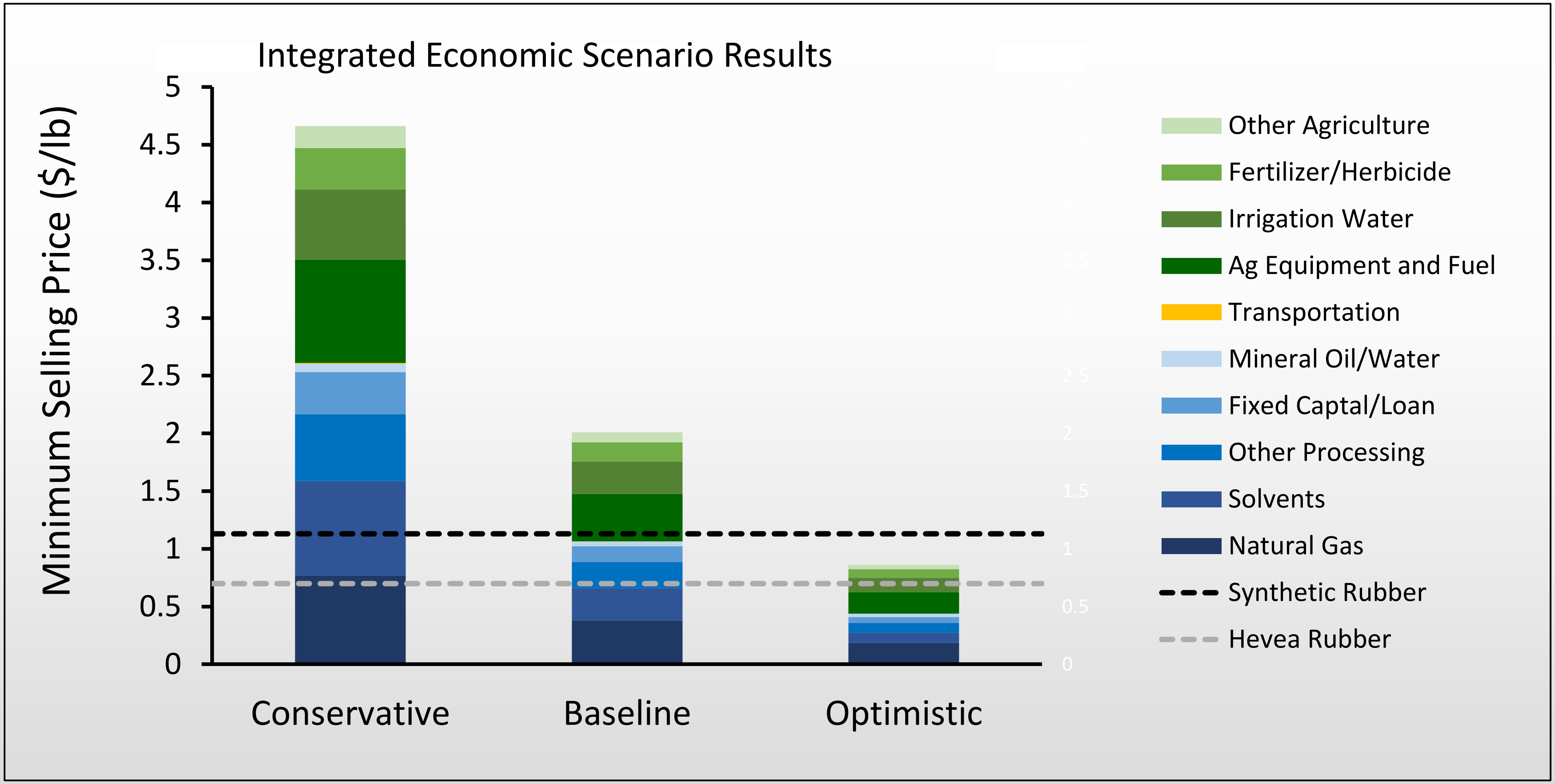
Baseline Integrated Economic Results - Guayule

Multiple scenarios completed

Example: bagasse revenue = $0.04 /lb, resin revenue = $1.00/lb



Multiple Scenarios Developed

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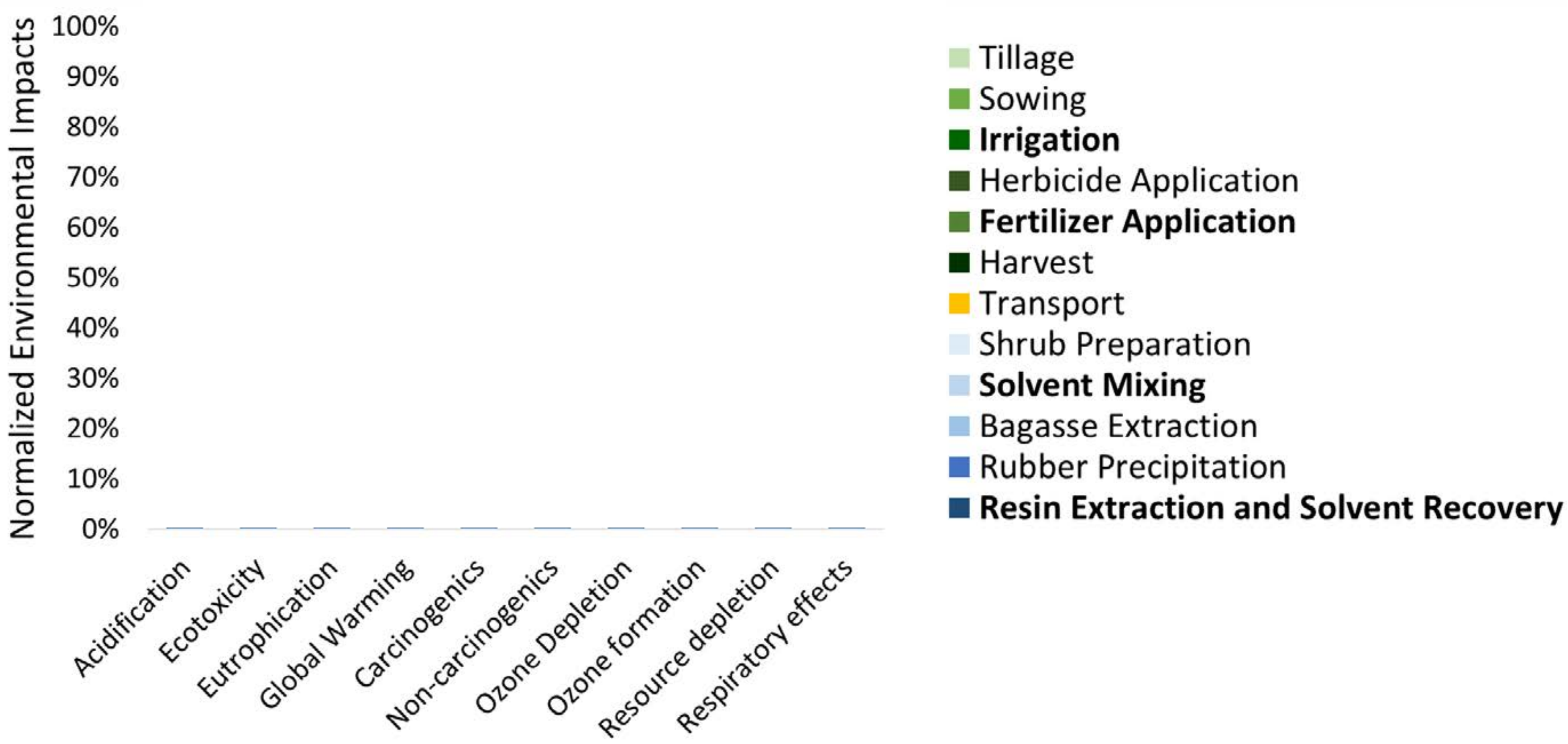
Baseline Integrated Environmental Results - Guayule



Environmental Impact Across 10 Impact Categories

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TEA & LCA Methods : Guar

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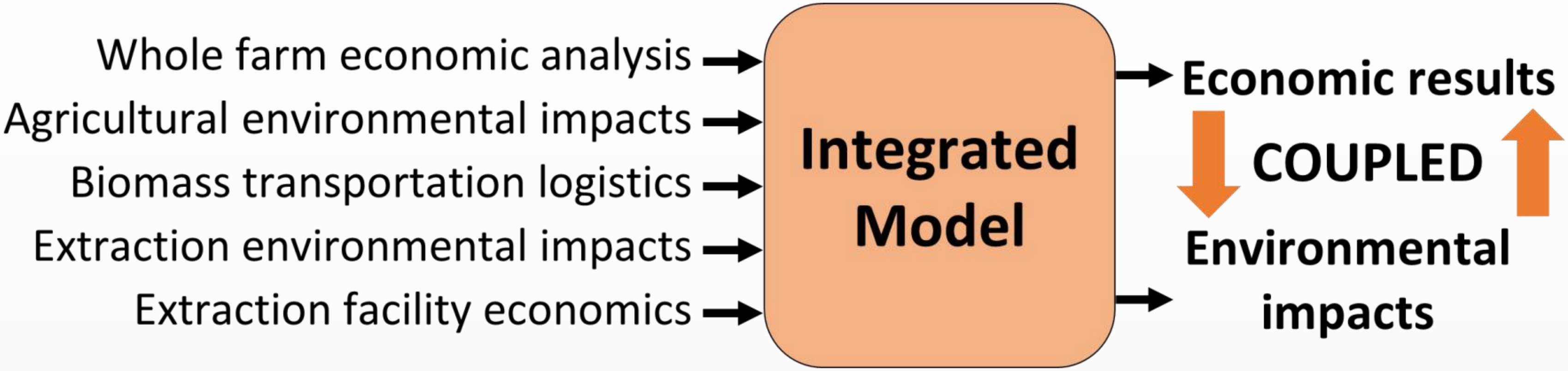

Whole farm economic analysis

Agricultural environmental impacts

Biomass transportation logistics

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Extraction facility economics

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Environmental impacts

Discounted Cash Flow Rate of Return

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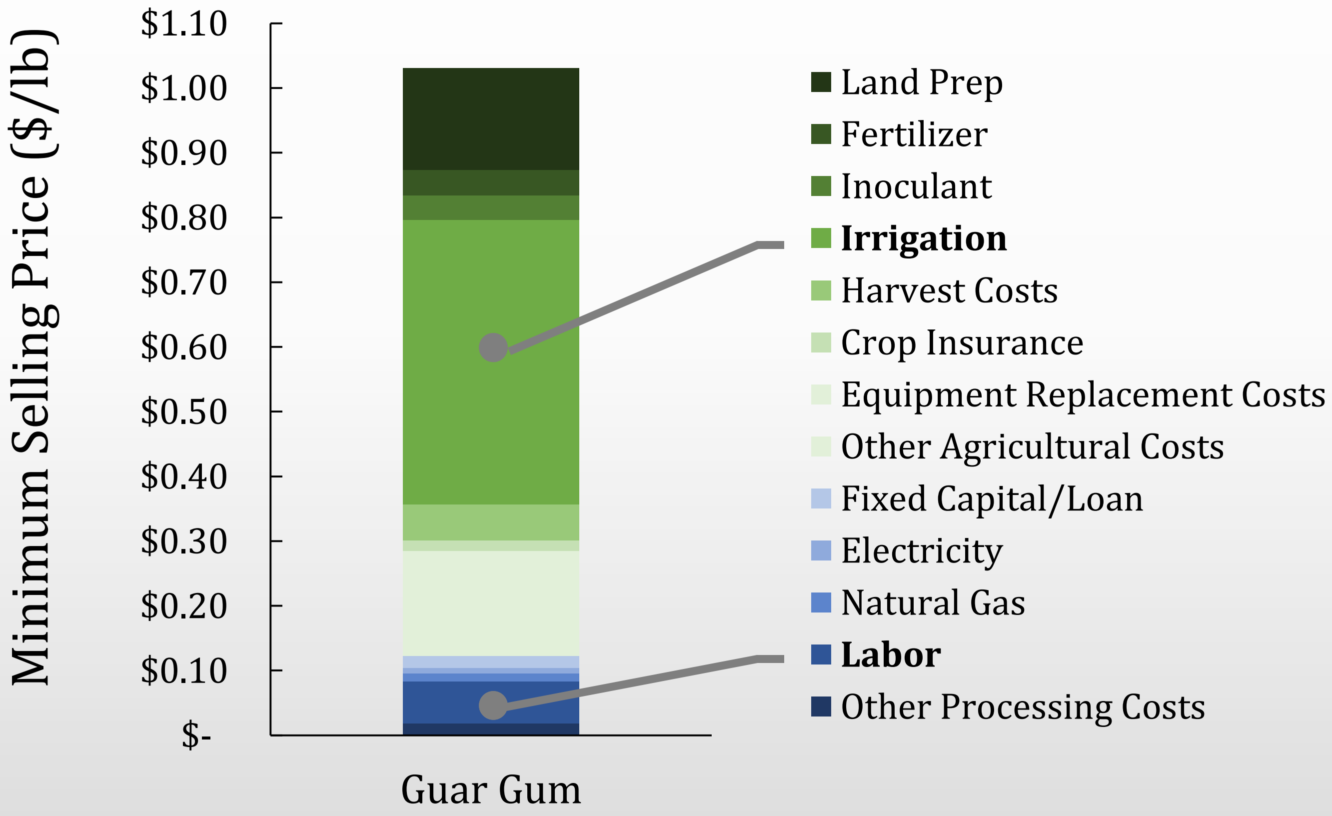
Baseline Integrated Economic Results - Guar



Robust Baseline Results: Highlighted impact areas

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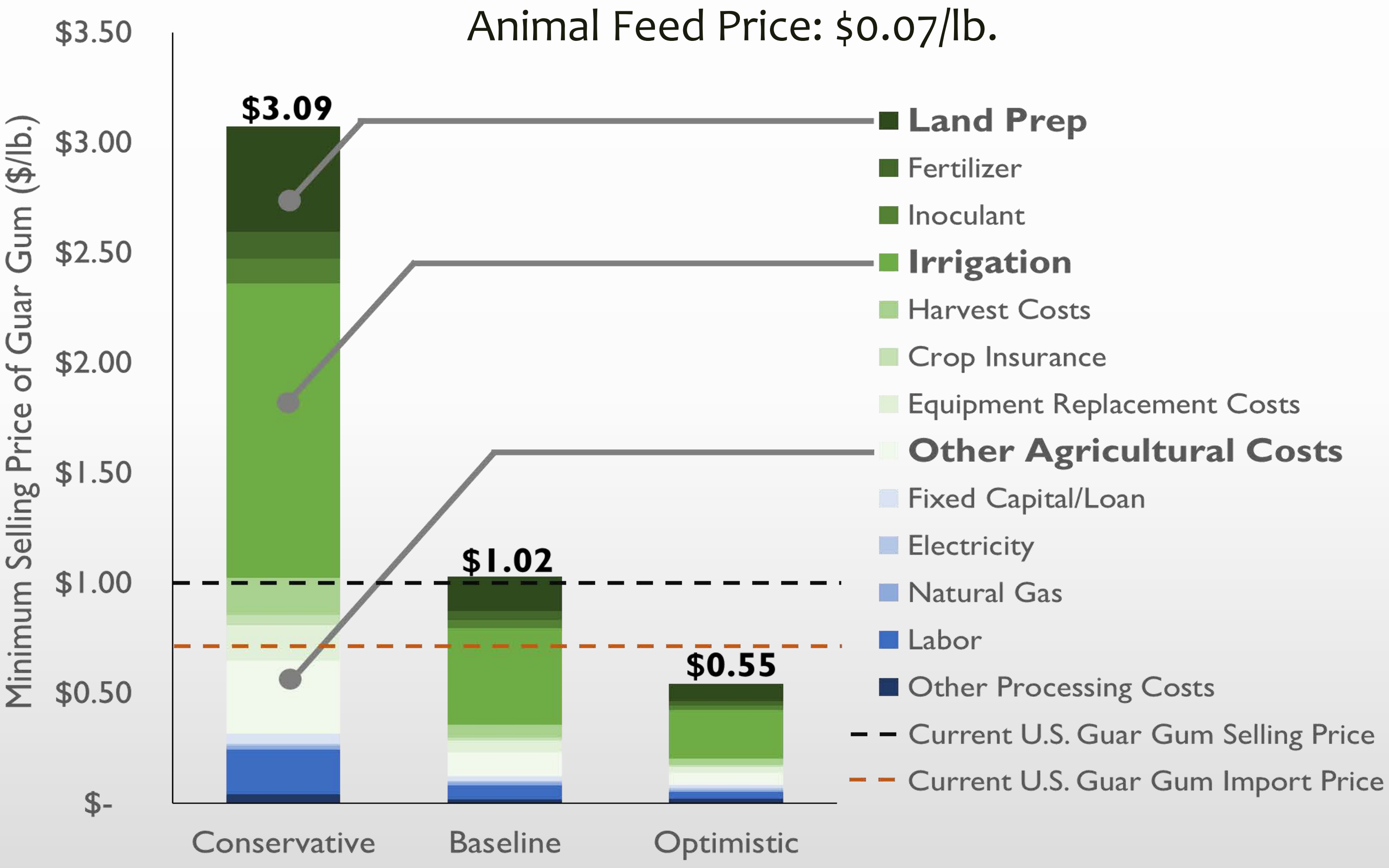
Baseline Integrated Economic Results - Guar

Multiple scenarios completed



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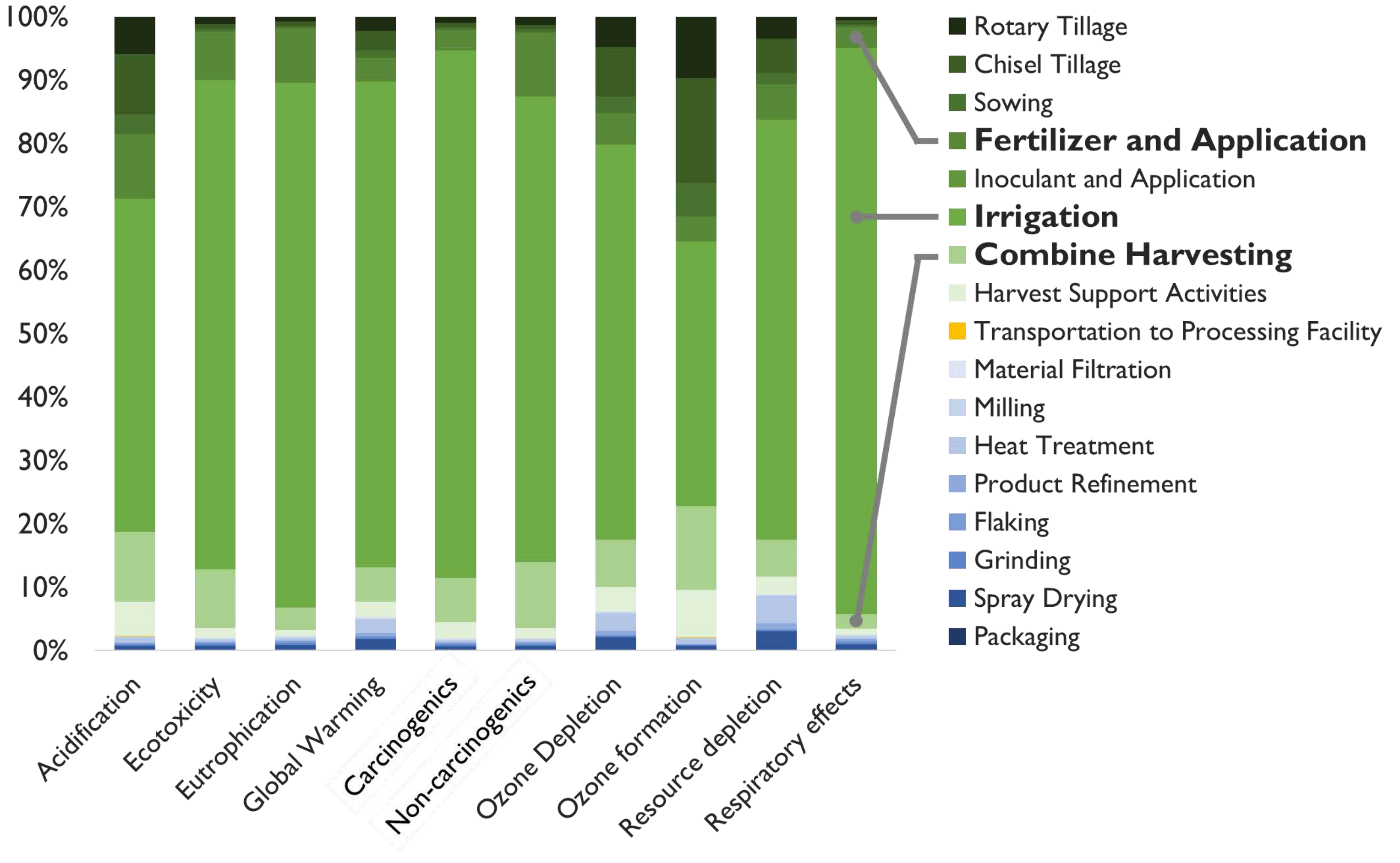
Baseline Integrated Environmental Results - Guar



Environmental Impact Across 10 Impact Categories

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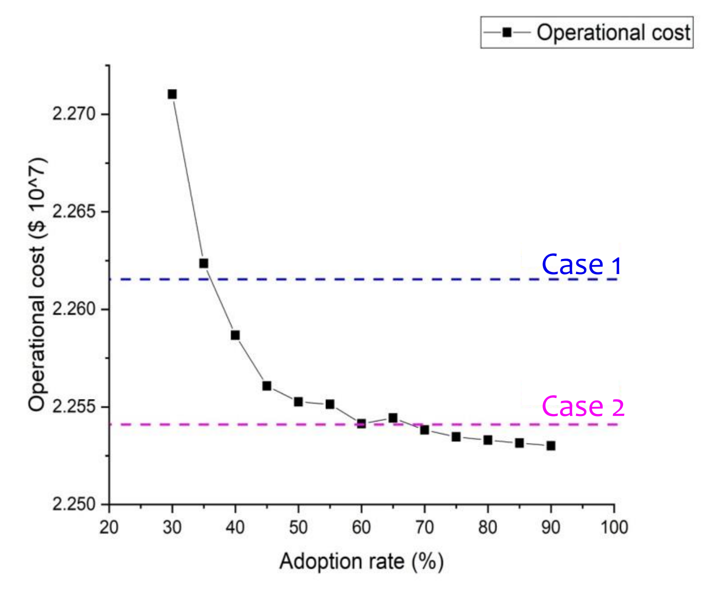
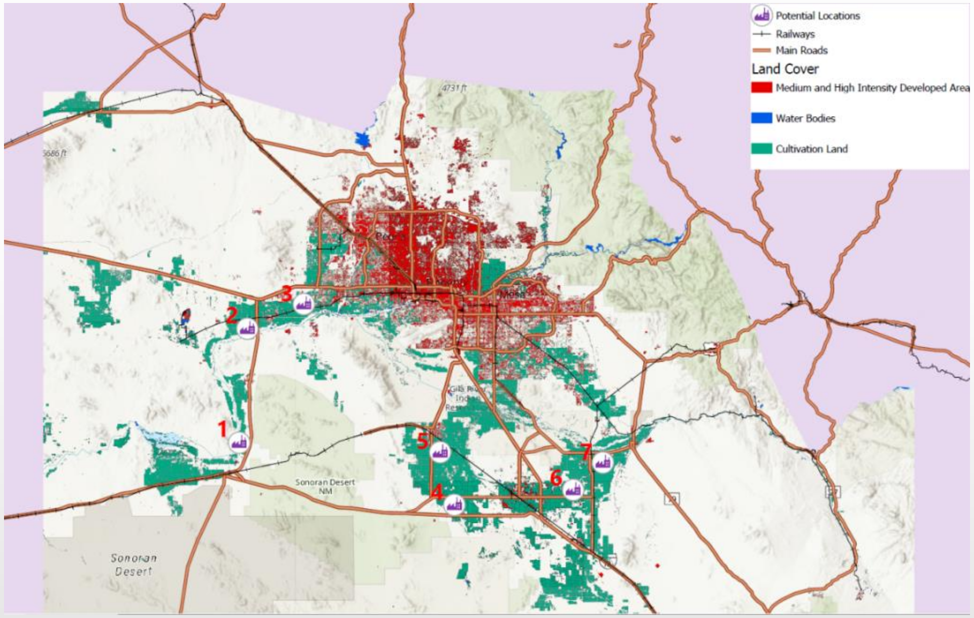
Distributed production and centralized processing

- Under different adoption rates of farms switching to Guayule, planning, operation and transportation costs change, as the average distance from field (farm) to process facility decreases



(case 1 and case 2 are stochastic cases, with different potential switching to grow Guayule)



The optimal process facility's location is identified as **location 5** in Pinal with capacity 431,109 metric tons of the process facility

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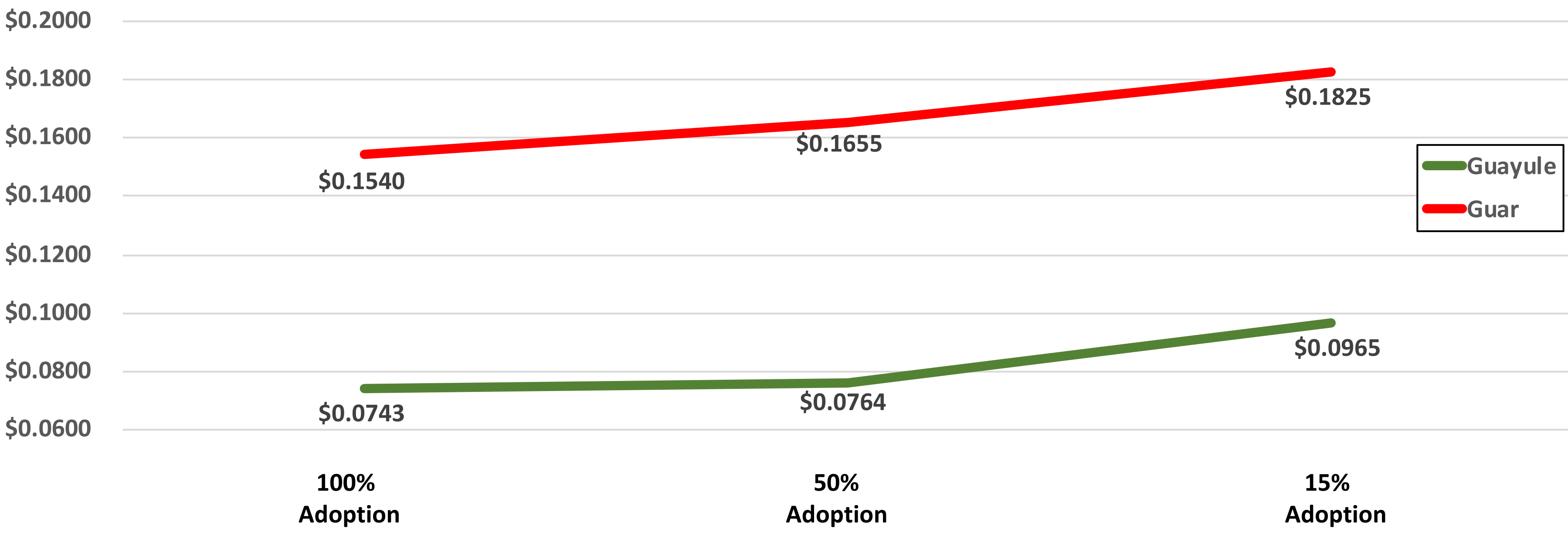
Breakeven Price to Grower Based on Adoption Rate into Current Cropping System $/pound



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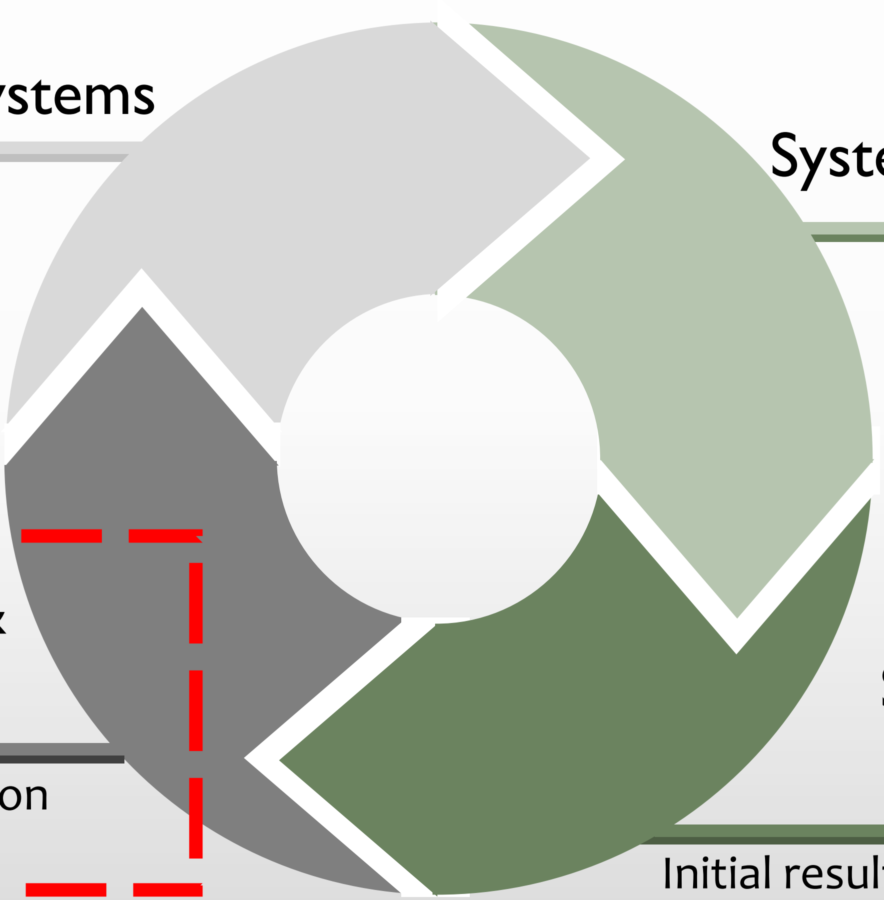
Sustainability Team



Experimental Systems

Identified high-impact targets

System Modeling

Integration complete: *Energy and Mass balances*

Sustainability Modeling

Initial results

- Costs/benefits of production
- Environmental Impacts

Data Feedback & Validation

De-risking production

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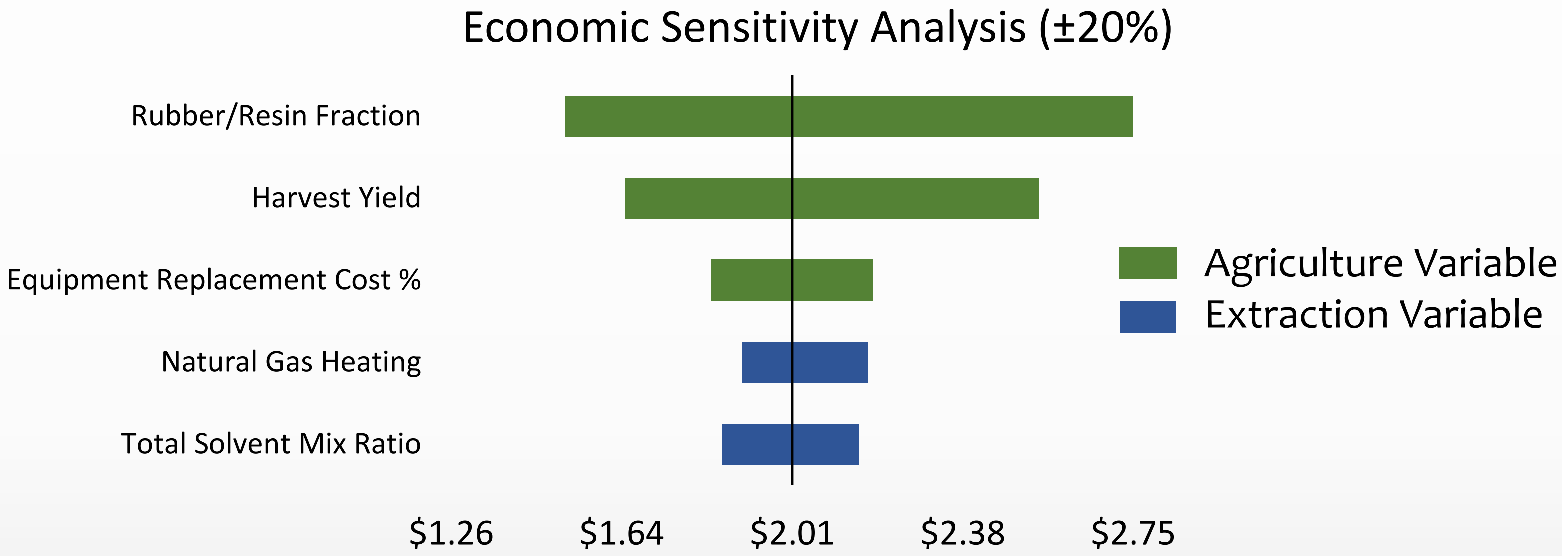
Sensitivity & Scenario Analysis -Guayule



LCA Sensitivity Top 5 Variables (avg. across 10 impact categories)
Rubber/Resin Fraction
Total Solvent Mix Ratio
Hexane Mix Ratio
Harvest Yield
Solvent left on rubber

Identifying critical input parameters

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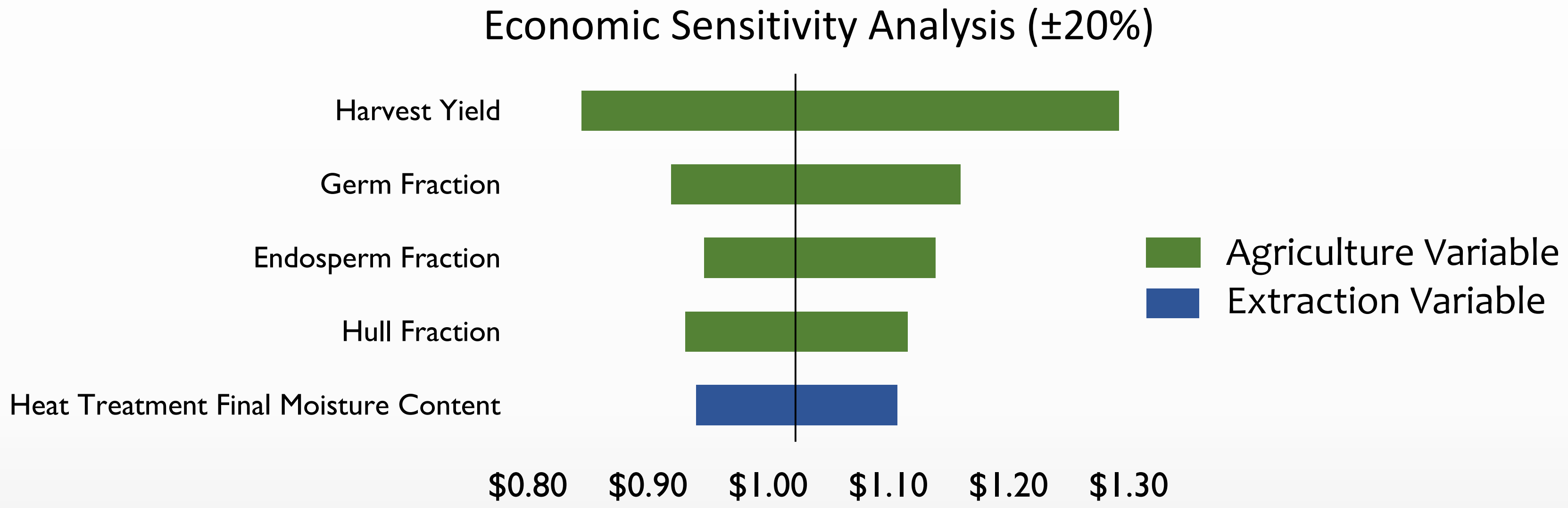
Sensitivity & Scenario Analysis -Guar



LCA Sensitivity Top 5 Variables (avg. across 10 impact categories)
Harvest Yield
Irrigation Water Amount
Germ Fraction
Endosperm Fraction
Hull Fraction

Identifying critical input parameters

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High Impact Variables

Guayule

- Rubber yield
- Irrigation
- Harvest yield
- Solvent
- Co-product value
- Downstream processing

Guar

- Harvest yield
- Irrigation
- Germ yield
- Moisture content
- Land Prep

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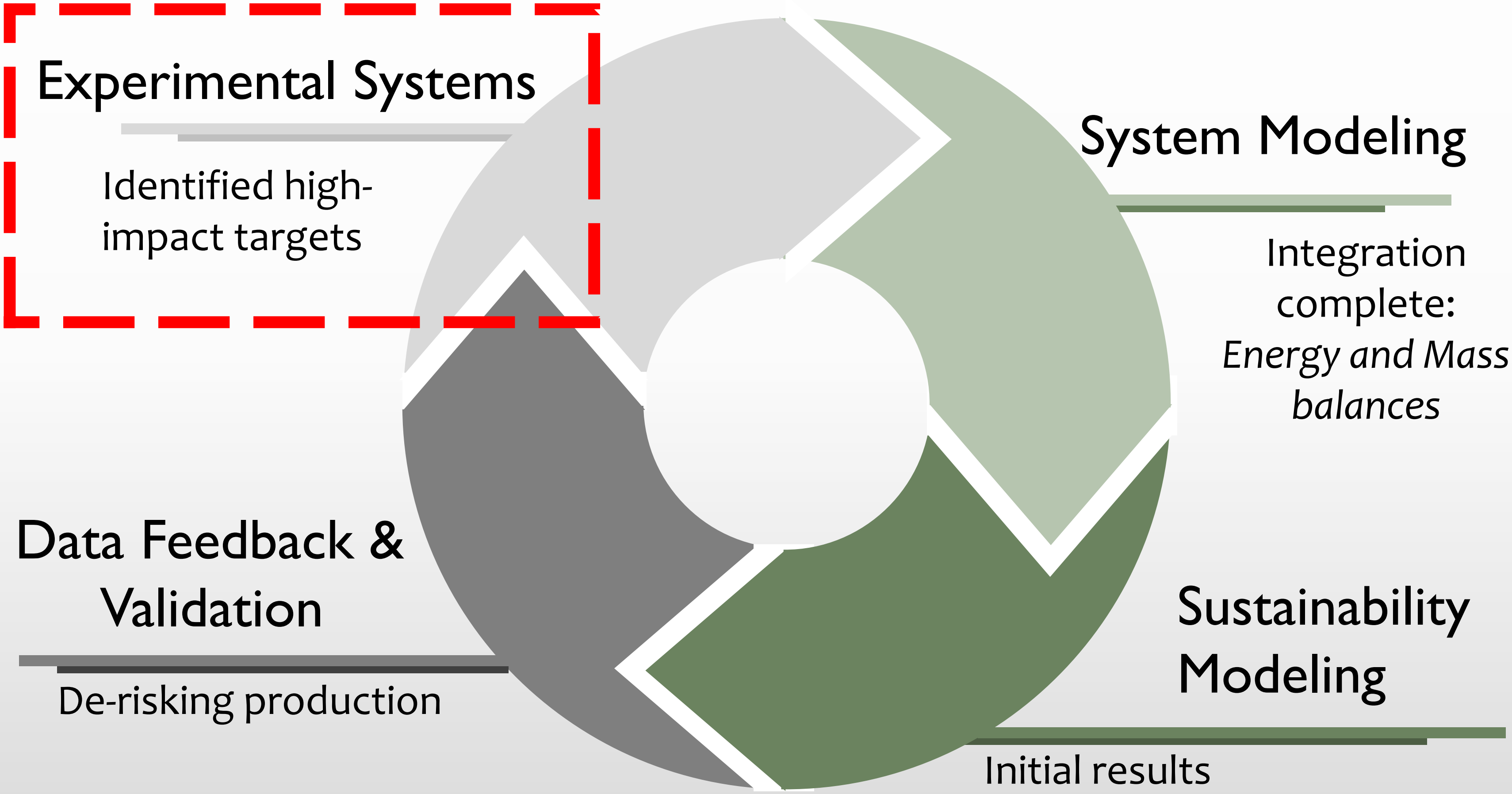
Sustainability Team



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Integrating field data into sustainability models

Goals & Actions

- Incorporate field data into sustainability models
  - *Send us a summary of the inputs to your field experiments!*
  - *to improve SBAR understanding of $cost and env hotspots*
- We will validate our use of your data via interviews with you to ensure model represents best practice
  - *Point of Contact: VeeAnder*
  - *Meet her!!*

Examples of data we need:

Guar & Guayule Field trials

All Inputs

All Outputs

Examples:
- Amount of water and type of irrigation
- Amount and type of fertilizer or herbicide
- Equipment type, hp, & hours

- Yields
- Field emissions

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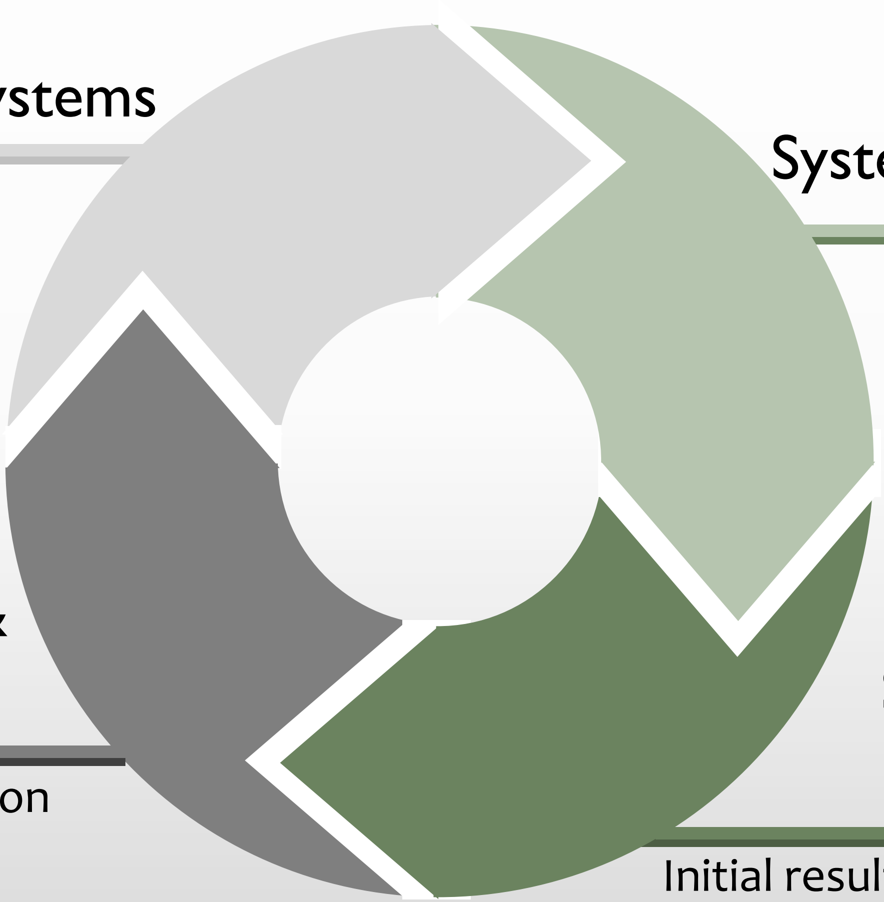
Sustainability Team: Year 3 Goals

United States Department of Agriculture

National Institute of Food and Agriculture



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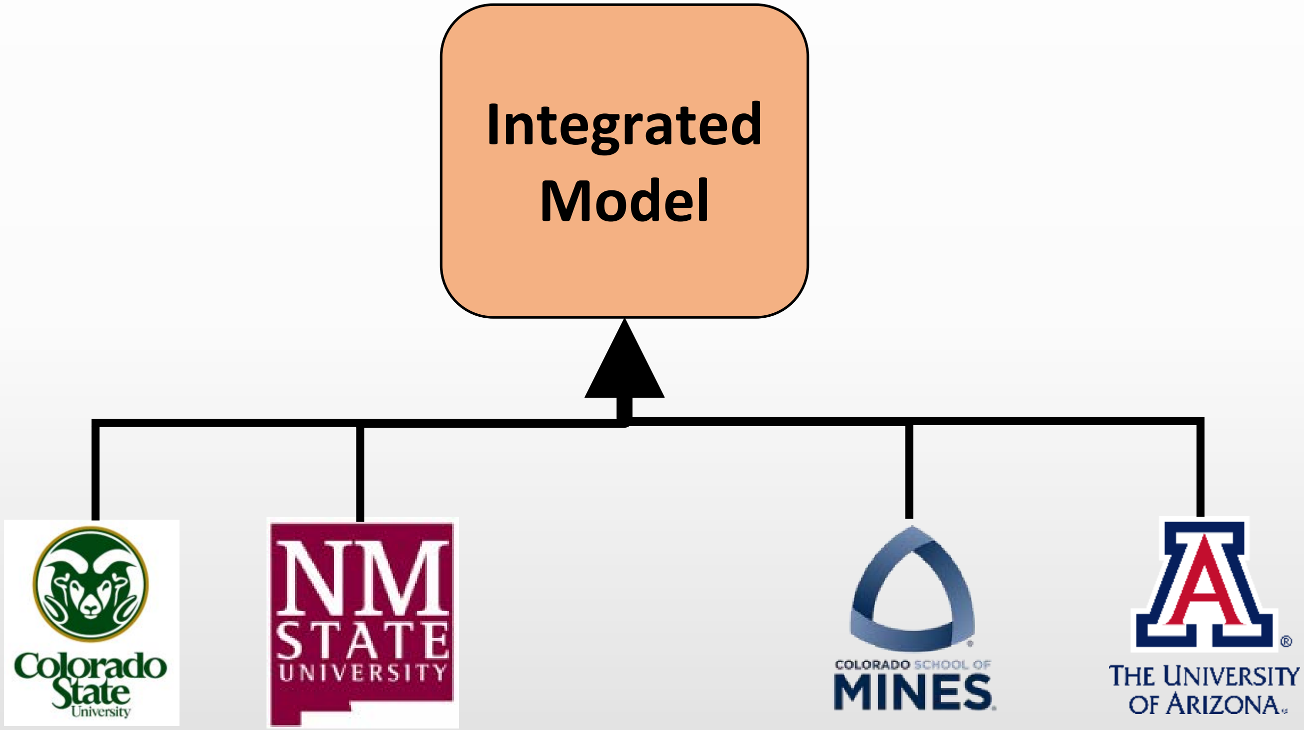
Individual University Year 3 Goals



Integrated Model

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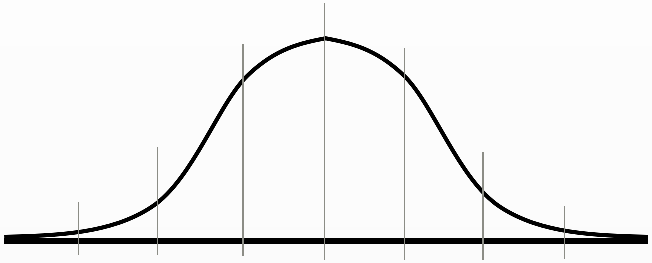
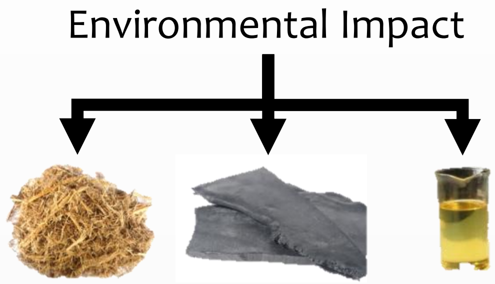
CSU Year 3 Objectives

Stochastic modeling with field data

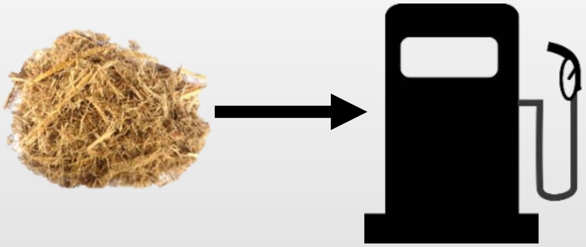


Allocation and displacement



Environmental Impact

Integrate bagasse to fuels modeling



Further characterize water consumption

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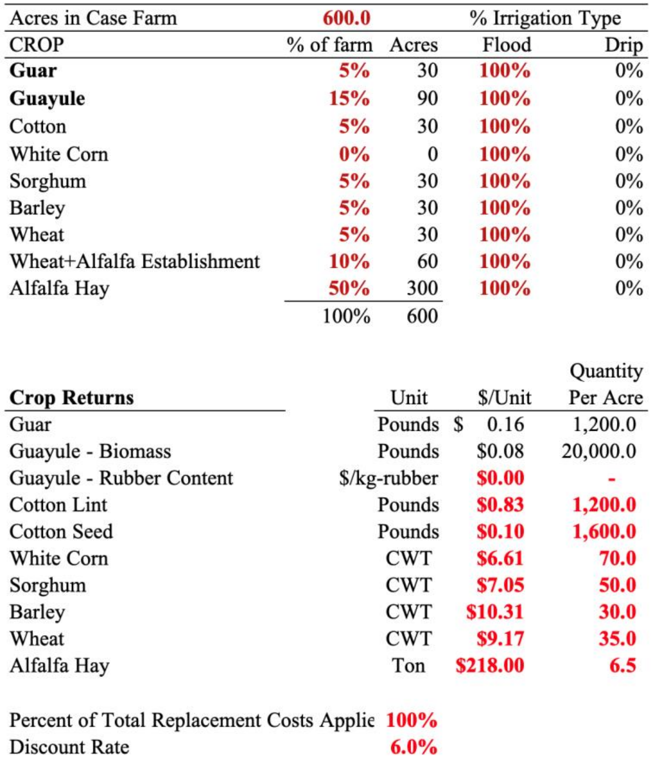
Farm Economic Team: Year 3 Objectives

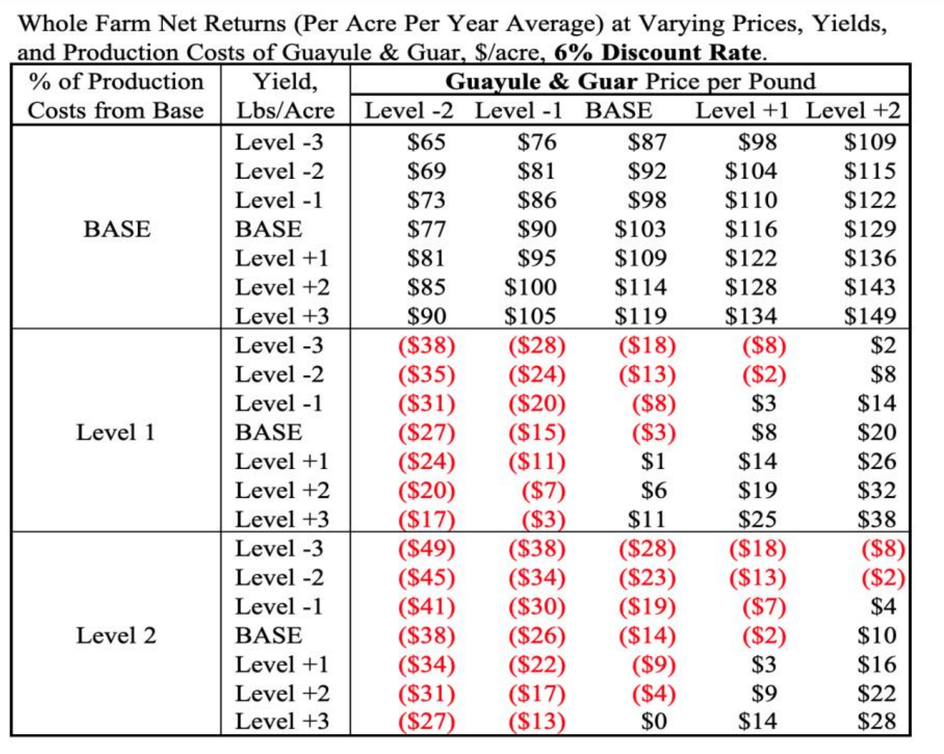
Total Farm Acres and Crop Returns



Acres in Case Farm	600.0		% Irrigation Type	
CROP	% of farm	Acres	Flood	Drip
Guar	5%	30	100%	0%
Guayule	15%	90	100%	0%
Cotton	5%	30	100%	0%
White Corn	0%	0	100%	0%
Sorghum	5%	30	100%	0%
Barley	5%	30	100%	0%
Wheat	5%	30	100%	0%
Wheat+Alfalfa Establishment	10%	60	100%	0%
Alfalfa Hay	50%	300	100%	0%
	100%	600		

Crop Returns	Unit	$/Unit	Quantity Per Acre
Guar	Pounds	$ 0.16	1,200.0
Guayule - Biomass	Pounds	$0.08	20,000.0
Guayule - Rubber Content	$/kg-rubber	$0.00	-
Cotton Lint	Pounds	$0.83	1,200.0
Cotton Seed	Pounds	$0.10	1,600.0
White Corn	CWT	$6.61	70.0
Sorghum	CWT	$7.05	50.0
Barley	CWT	$10.31	30.0
Wheat	CWT	$9.17	35.0
Alfalfa Hay	Ton	$218.00	6.5

Percent of Total Replacement Costs Applie	100%
Discount Rate	6.0%



Whole Farm Net Returns (Per Acre Per Year Average) at Varying Prices, Yields, and Production Costs of Guayule & Guar, $/acre, **6% Discount Rate**.

% of Production Costs from Base	Yield, Lbs/Acre	**Guayule & Guar** Price per Pound				
		Level -2	Level -1	BASE	Level +1	Level +2
BASE	Level -3	$65	$76	$87	$98	$109
	Level -2	$69	$81	$92	$104	$115
	Level -1	$73	$86	$98	$110	$122
	BASE	$77	$90	$103	$116	$129
	Level +1	$81	$95	$109	$122	$136
	Level +2	$85	$100	$114	$128	$143
	Level +3	$90	$105	$119	$134	$149
Level 1	Level -3	($38)	($28)	($18)	($8)	$2
	Level -2	($35)	($24)	($13)	($2)	$8
	Level -1	($31)	($20)	($8)	$3	$14
	BASE	($27)	($15)	($3)	$8	$20
	Level +1	($24)	($11)	$1	$14	$26
	Level +2	($20)	($7)	$6	$19	$32
	Level +3	($17)	($3)	$11	$25	$38
Level 2	Level -3	($49)	($38)	($28)	($18)	($8)
	Level -2	($45)	($34)	($23)	($13)	($2)
	Level -1	($41)	($30)	($19)	($7)	$4
	BASE	($38)	($26)	($14)	($2)	$10
	Level +1	($34)	($22)	($9)	$3	$16
	Level +2	($31)	($17)	($4)	$9	$22
	Level +3	($27)	($13)	$0	$14	$28

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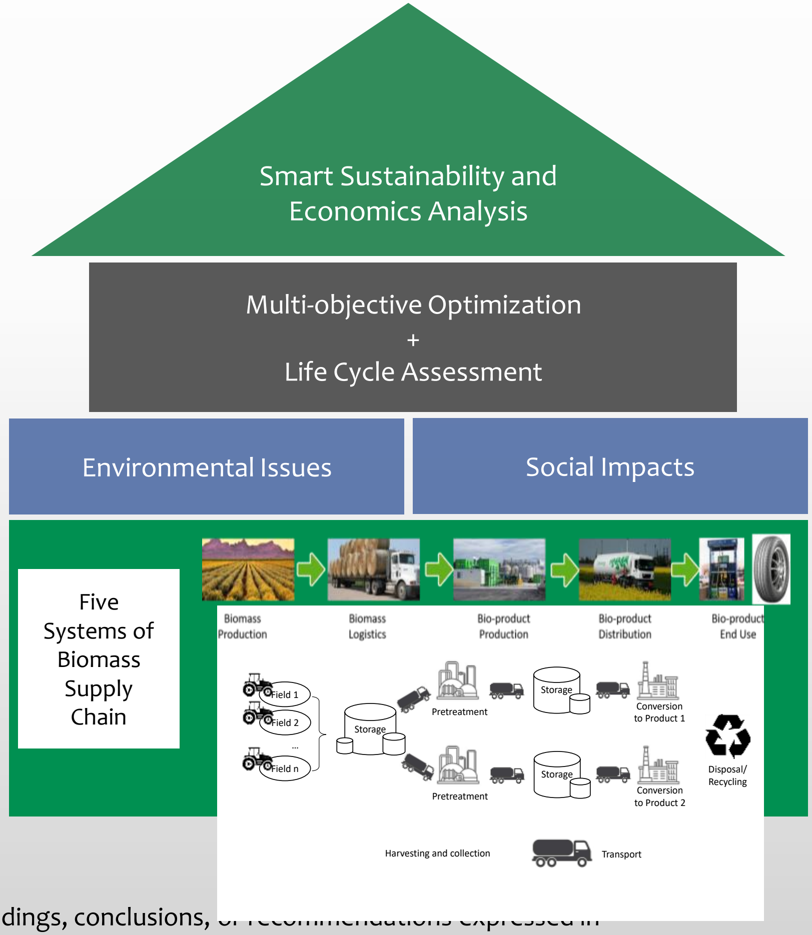
UofA Year 3 Objectives

Model and Algorithm Development for Biomass Supply Chain – Year 3 Tasks (Fan Group@UA)

- Perform more comprehensive sustainability and regional economics analysis, based on current data obtained from industrial partners, literature and sustainability group
  - *minimize the total annual capital and operation cost*
  - *minimize the total annual CO2-equivalent GHG emission*
  - *maximize the accrued local jobs*
- Apply integer optimization approaches to design the smart farm production plan and scheduling



Smart Sustainability and Economics Analysis

Multi-objective Optimization
+
Life Cycle Assessment

Environmental Issues

Social Impacts

Five Systems of Biomass Supply Chain

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Ag Model Year 3 Objectives

Establish **SOPs** for transferring field data to ag sustainability model

Analyze **field data**, create stochastic model

Identify **hot spots** (I.e. areas for improvement) and feed results & recommendations back to research team

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Mines Year 3 Objectives

Develop social sustainability indicators for guar & guayule

- From IMPLAN in collaboration with econ team
- From information mined from your research and extension activities!
- Contact us if you have social sustainability ideas or data!

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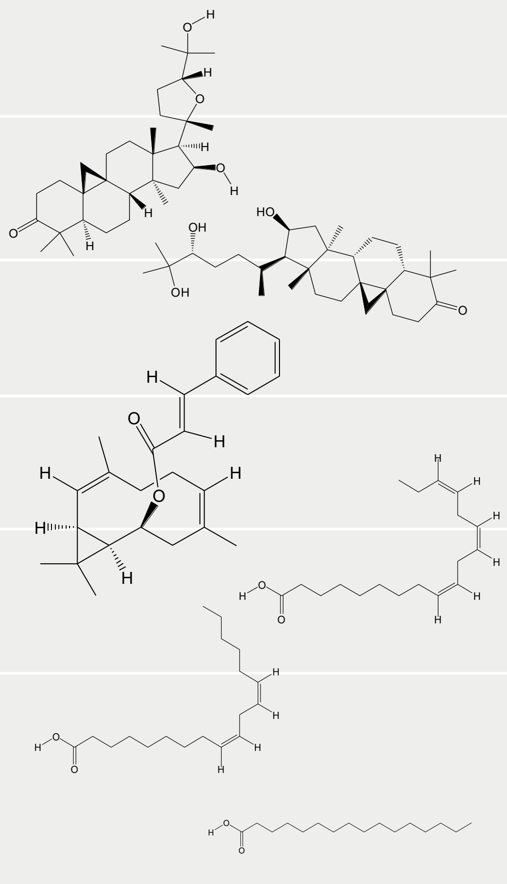
United States Department of Agriculture
National Institute of Food and Agriculture

NMSU Year 3 Objectives

Bagasse & Resin Utilization – Year 3 (Brewer & Jena Groups @NMSU)



Argentatin A $(C_{30}H_{48}O_4)$	
Argentatin C $(C_{30}H_{50}O_4)$	
Guayulin A $(C_{24}H_{30}O_2)$	
Linoleic acid $(C_{18}H_{32}O_2)$	
Linolenic acid $(C_{18}H_{30}O_2)$	
Palmitic acid $(C_{16}H_{32}O_2)$	

- Guayule resin characterization and applications review
- Low-cost, high-N biomass feedstock review
- Low-cost, high-N biomass biochemical conversion review

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Year 2: Accomplishments

Objectives
1: Fully integrate model
2: Results for environmental impact
3: Results for economic impact
4: Evaluation of distributed versus local processing
5: Data feedback for de-risking technology

- Student Awards
  - ISSST: 1st place poster winner
  - ACLCA: 1st place poster winner
  - Sussman Fellowship: Summer internship support
- Graduates
  - Sun, Robbs

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Year 3: Goals

Our project mission is to build a sustainable bio-economy for arid regions to improve quality of life in rural communities and Native Nations.

- Data Integration
- Logistics optimization across multiple indicators
- Improved model fidelity
- Social Sustainability
- Extension support
- Data feedback to SBAR team

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EXTENSION & OUTREACH

***LEADS**: John Idowu (NM), Blase Evancho (AZ)*

***Team**: Angadi, Begna, Bhandari, Brassill, Darapuneri, Dierig, Duncan, Fields, Garcia, Gellis, Grover, Gutierrez, Khanal, Miller, Ogden, Paquin, Prieto, Pruitt, Rock, Rodriguez-Uribe, Rogstad, Salinas, Sikora, Singh J., Singh P., Skelton, Teegerstrom, Wang*

Sustainable Bioeconomy for Arid Regions
Grower-Focused Extension & 4-H


Guar Field in NM


4-H Workshop


Guayule Field in AZ

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SBAR – Extension Grower-Focused/4-H

Main Objectives of this group

- 5.1 Produce Extension bulletins and web materials to inform growers of agronomic and irrigation requirements. Conduct needs assessment of growers
- 5.2 Hold workshops throughout the region on sustainable practices to expand crop production to new rural regions and Native Nation lands. Use existing meeting to introduce project.
- 5.5 Involve youth in 4-H projects and STEM summer camps.

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Key Contributors

Leads/Co-PIs

- Blase Evancho - U of A
- Dr. Channah Rock – U of A
- Dr. Paul Gutierrez – NMSU
- Dr. Kulbhushan Grover – NMSU
- Dr. Sangu Angadi – NMSU
- Dr. John Idowu – NMSU

Staff members:

- Dr. Laura Rodriguez-Uribe
- Natalie Brassill
- Cara Duncan
- Torran Anderson

Collaborators:

- Dr. Murali Darapuneni
- Dr. Sultan Begna

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Students

Student	Advisor
Darien Pruitt	Dr. John Idowu
Paramveer Singh	Dr. Sangu Angadi
Jagdeep Singh	Dr. Sangu Angadi
Alonso Garcia	Dr. Kulbhushan Grover

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SBAR

USDA United States Department of Agriculture National Institute of Food and Agriculture

Changes Made During Year 2

- Addition of Blase Evancho – Strengthen Grower Extension in Arizona
- 4-H in Arizona to be more directed towards counties, especially the native Americans and underserved communities
- Retreat of EEO group held on December 5-7, 2018 @NMSU
  - Project Evaluator was able to meet with each subgroup
  - Emphasis on proper documentation of events and activities being conducted through the project
  - Collaboration across different subgroups was strengthened
- New Collaborators and students added to SBAR-EEO group.

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Achievements during Year 2

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Arizona Cooperative Extension -

Channah Rock, Natalie Brassill, & Stevi Zozaya

- 4 Project Puente interns participated in SBAR specific projects
- Recruitment from different counties - Tucson, Pima and Pinal counties
- Completed stakeholder needs assessment for Guar

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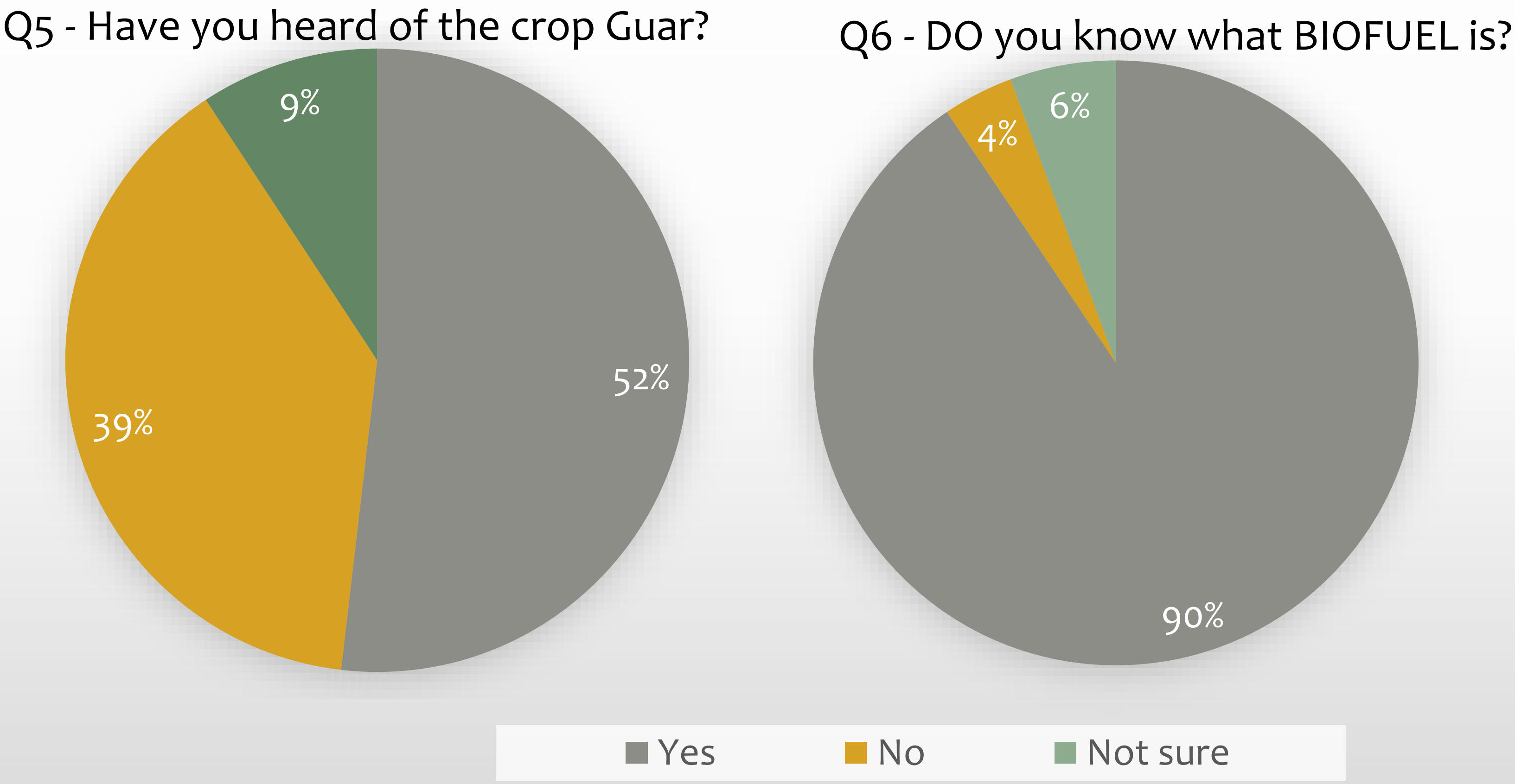
TERMINOLOGY AND AWARENESS



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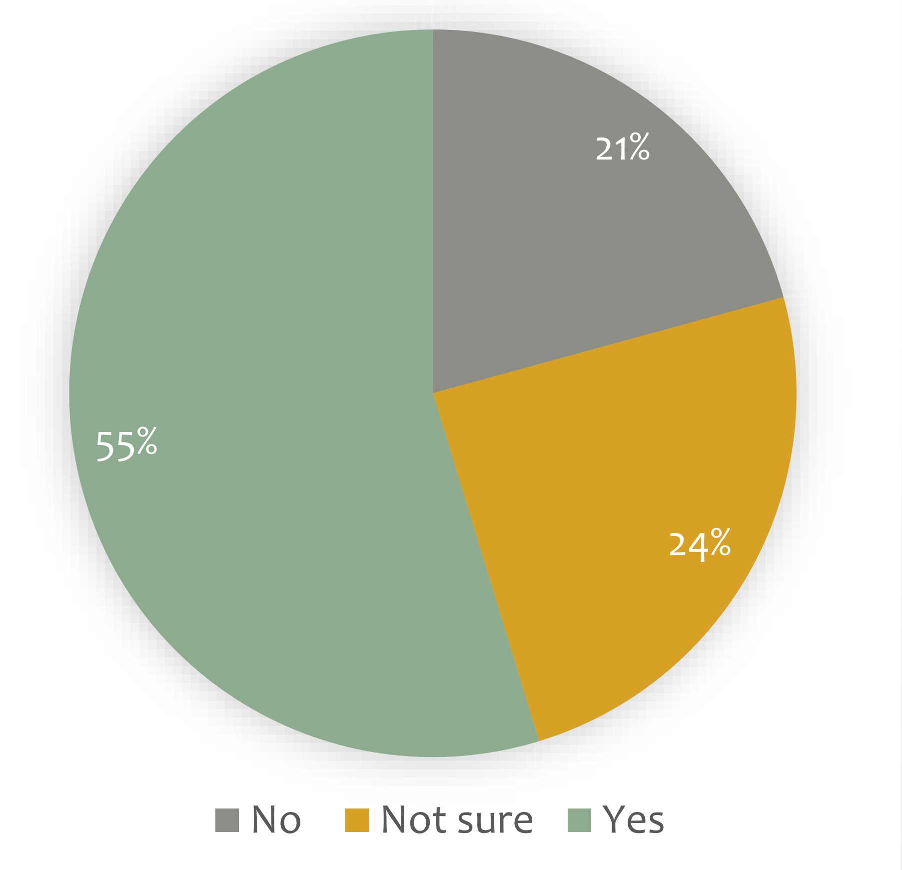
Sustainability Ethic

Q8 - Some suggested benefits of the production of biofuel and bio-product crops include promoting America's energy security, reduction in greenhouse gas emissions, protection of the environment, and financial benefits for farmers and agricultural businesses.

Taking that into consideration, are you interested in the **production of biofuel and bio-product crops** as a sustainable resource?



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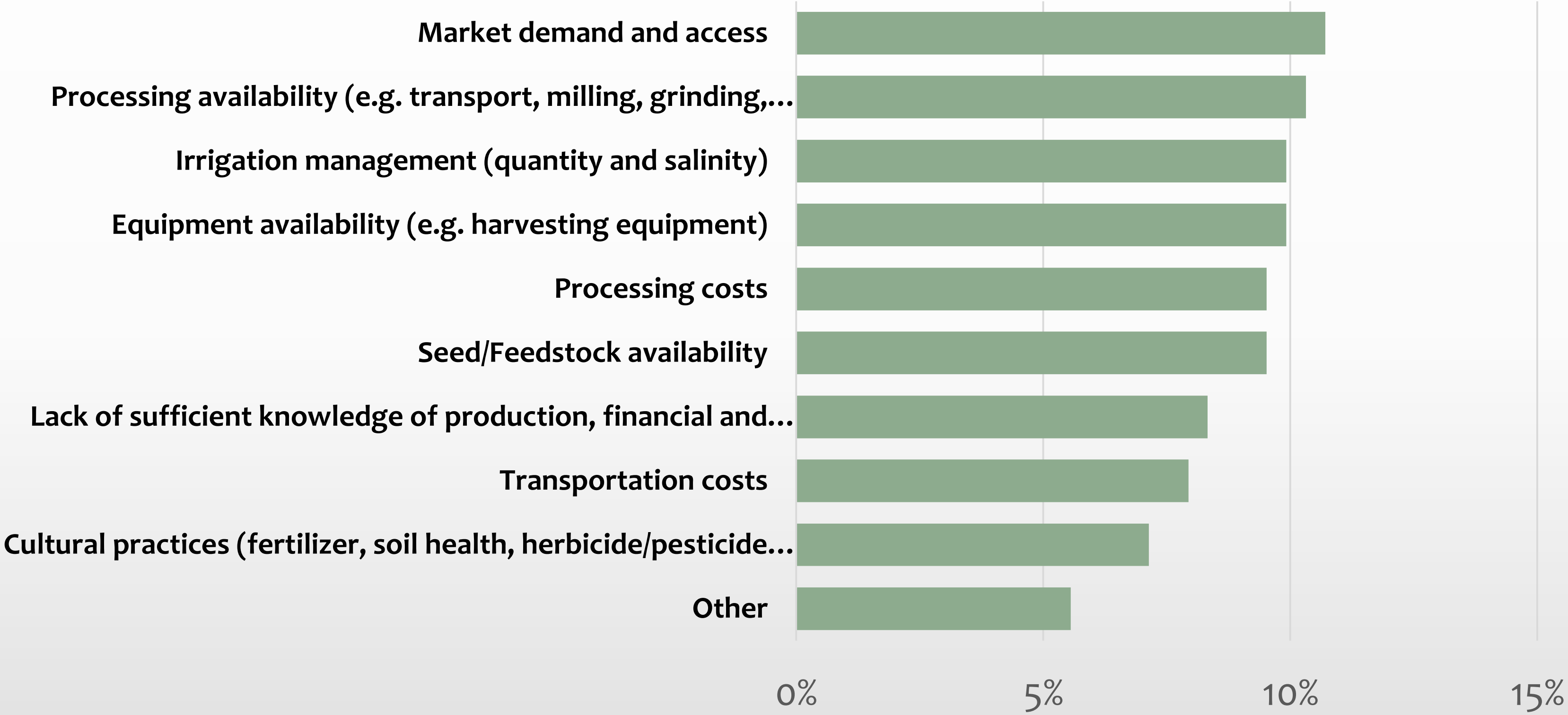
Questions/Concerns



"Other" - Water costs; Benefits of the product …

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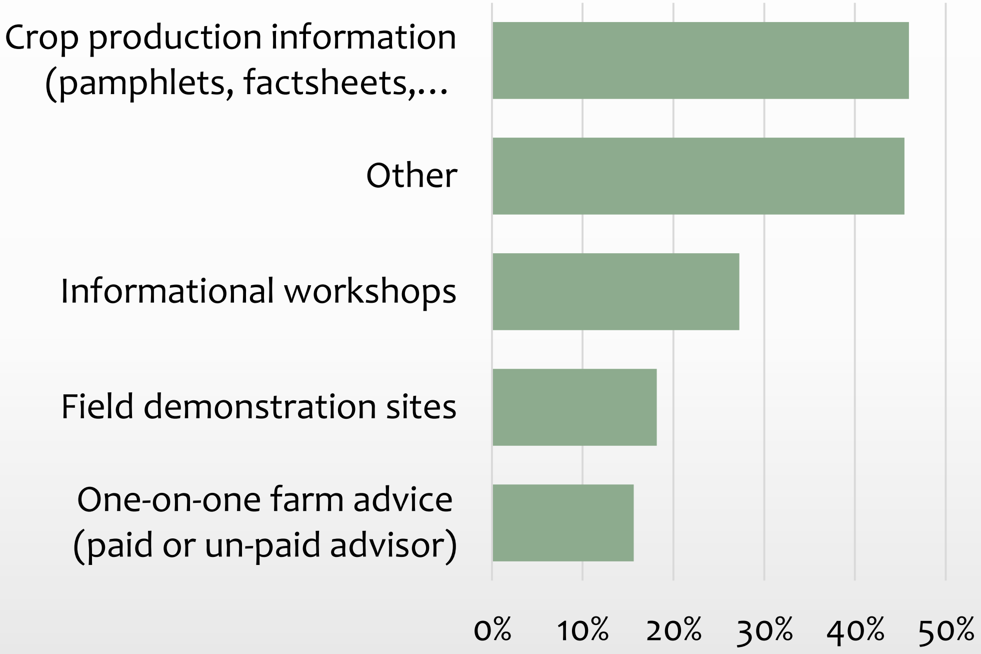
How do you want information?

Q10 - What would help answer your questions regarding biofuel or bio-product crops such as Guar? (rank in order of most helpful (1) to least helpful (5)

"Other"

- Talking to a processor
- Talk to farmers who have experience growing guar
- Talk to a grower



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Arizona Extension

- Presented guayule information and research topics at several events.
  - 11/16/18 San Carlos Reservation Visit
  - 12/14/18 GRIC Farm Board Meeting
  - 1/10/19 Marana Winter Field Crops Meeting
  - 1/15/19 Casa Grande Winter Field Crops Meeting
  - 5/30/19 Bridgestone Guayule Tour

BRIDGESTONE GUAYULE TOUR

Market Discussion & Field Day
May 30, 2019

BRIDGESTONE
Worldwide Olympic Partner

Bridgestone Guayule Research Farm
4140 West Harmon Road
Eloy, AZ 85131

9:30-9:45am	Registration
9:45-10:15am	Dr. Dave Dierig - Guayule Market & Demand Discussion
10:15-10:40am	Dr. Dave Dierig – Farm Tour
10:40-11:00am	Dr. Sam Wang - Agronomic Research
11:00-11:20am	Dr. Bill McCloskey – Weed Management Strategies
11:20-11:30am	Dr. Peter Ellsworth – Insect Management Discussion
11:30-11:35am	Ms. Alix Rogstad – SBAR Description & Goals
11:35-12:00pm	Blase Evancho – Grower Discussion w/ 2 growers with active fields.
12:30-2:00pm	Travel to Rubber Extraction Facility

Bridgestone Guayule Rubber Extraction Facility
6533 South Mountain Road
Mesa, AZ 85212

2:00-3:15pm	Bob White – Tour of Facility & Extraction Process
3:15-3:30pm	Guayule Wrap-up

SUSTAINABLE BIOECONOMY FOR ARID REGIONS

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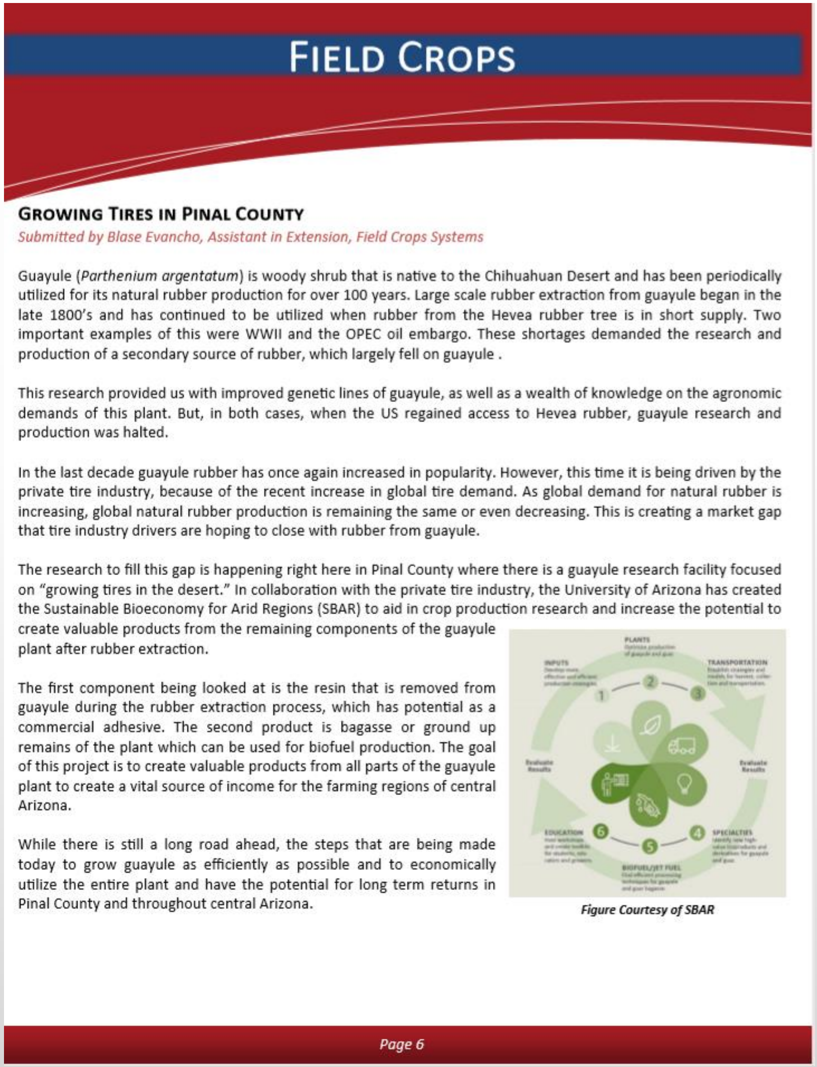
Arizona Extension

- SBAR needs assessment altered for Native American Communities and distributed.
- Guayule article distributed through Central Arizona Extension Newsletter "Crop Rotator" (200 subscribers)
- 2nd article Distributed through Pinal County Cooperative Extension quarterly newsletter (300 subscribers)



FIELD CROPS

GROWING TIRES IN PINAL COUNTY

Submitted by Blase Evancho, Assistant in Extension, Field Crops Systems

Guayule (*Parthenium argentatum*) is woody shrub that is native to the Chihuahuan Desert and has been periodically utilized for its natural rubber production for over 100 years. Large scale rubber extraction from guayule began in the late 1800's and has continued to be utilized when rubber from the Hevea rubber tree is in short supply. Two important examples of this were WWII and the OPEC oil embargo. These shortages demanded the research and production of a secondary source of rubber, which largely fell on guayule .

This research provided us with improved genetic lines of guayule, as well as a wealth of knowledge on the agronomic demands of this plant. But, in both cases, when the US regained access to Hevea rubber, guayule research and production was halted.

In the last decade guayule rubber has once again increased in popularity. However, this time it is being driven by the private tire industry, because of the recent increase in global tire demand. As global demand for natural rubber is increasing, global natural rubber production is remaining the same or even decreasing. This is creating a market gap that tire industry drivers are hoping to close with rubber from guayule.

The research to fill this gap is happening right here in Pinal County where there is a guayule research facility focused on "growing tires in the desert." In collaboration with the private tire industry, the University of Arizona has created the Sustainable Bioeconomy for Arid Regions (SBAR) to aid in crop production research and increase the potential to create valuable products from the remaining components of the guayule plant after rubber extraction.

The first component being looked at is the resin that is removed from guayule during the rubber extraction process, which has potential as a commercial adhesive. The second product is bagasse or ground up remains of the plant which can be used for biofuel production. The goal of this project is to create valuable products from all parts of the guayule plant to create a vital source of income for the farming regions of central Arizona.

While there is still a long road ahead, the steps that are being made today to grow guayule as efficiently as possible and to economically utilize the entire plant and have the potential for long term returns in Pinal County and throughout central Arizona.

Figure Courtesy of SBAR

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Events in New Mexico

Event	Number of Contacts
NM Sustainable Agriculture Conference (December, 2018)	**100**
NM Cotton Conference January, 2019	**100**
NM Organic Farming Conference February, 2019	**1,100**
NM Sustainable Agriculture Field Day June, 2019	**55**
Field Day at Clovis Ag. Science Center August, 2019	**150**
Field Day at Tucumcari Ag Science Center August, 2019	**110**
Field Day at Los Lunas Ag. Science Center August, 2019	**150**

- **A farmer committed to growing guar next year in Clovis area**

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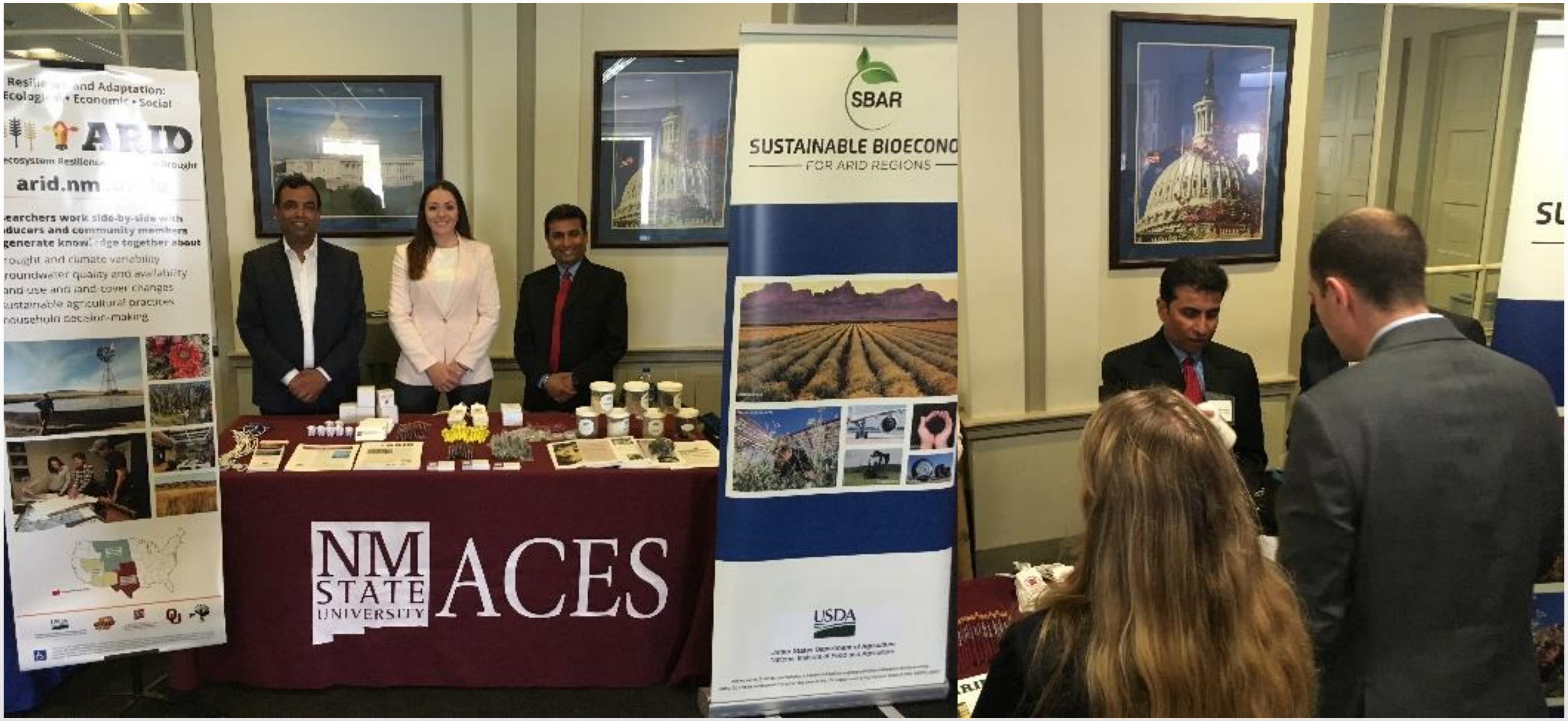
SBAR Extension/Outreach



Presentation of SBAR Project at US Congressional Research Exhibit, Washington DC.

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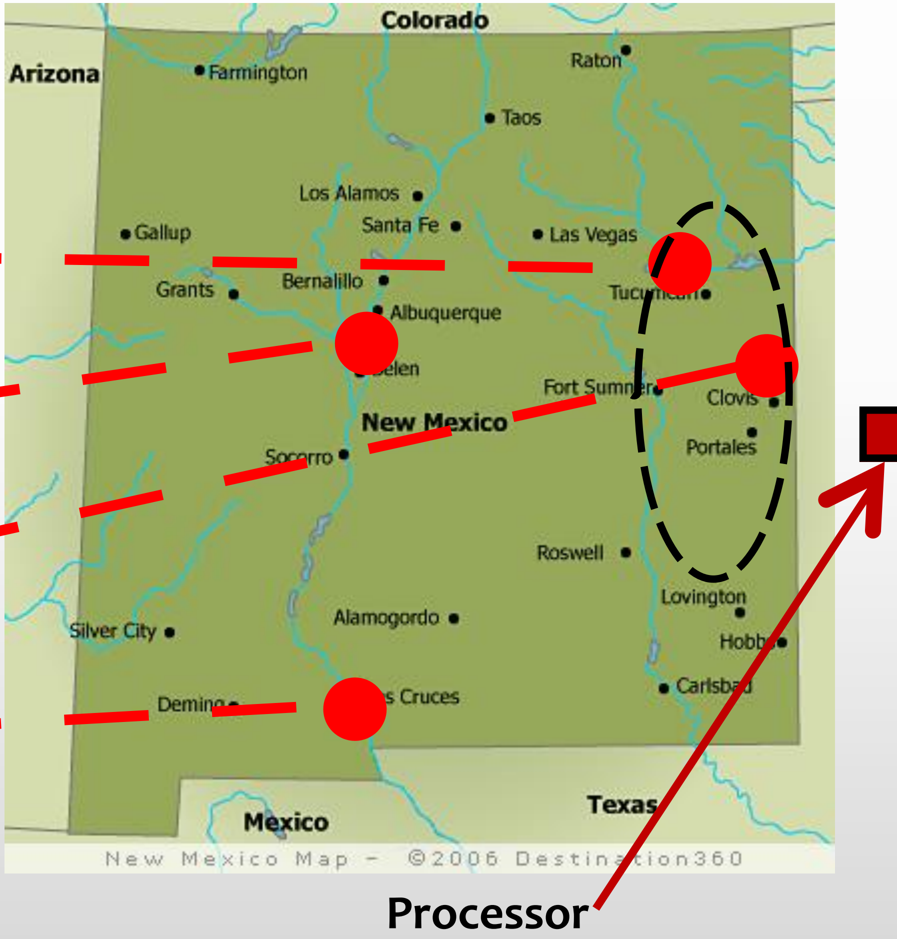
On-station demonstration trial 2019

- Nitrogen and Phosphorus Response
- Trials at
  - NMSU Agricultural Science Center in Tucumcari
  - NMSU Agricultural Science Center in Los Lunas
  - NMSU Agricultural Science Center in Clovis
  - NMSU Leyendecker Plant Science Center Las Cruces

Field Days Held at Places Highlighted in Yellow



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SBAR

USDA
United States
Department of
Agriculture

National Institute
of Food and
Agriculture

2019 SBAR Guar Demonstration Trials (NM)

Las Cruces

Clovis

Los Lunas

Tucumcari

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2018 On-station Demonstration Trials

- On-station Inoculum and Phosphorus Response Demonstration Trials with Guar (2018) set up at:
  - NMSU Agricultural Science Center in Los Lunas
  - NMSU Agricultural Science Center in Clovis

- 2018 Yields
  - *Los Lunas – 800 lb/ac*
  - *Clovis – 1000lb/ac*

Guar Field in Los Lunas 2018

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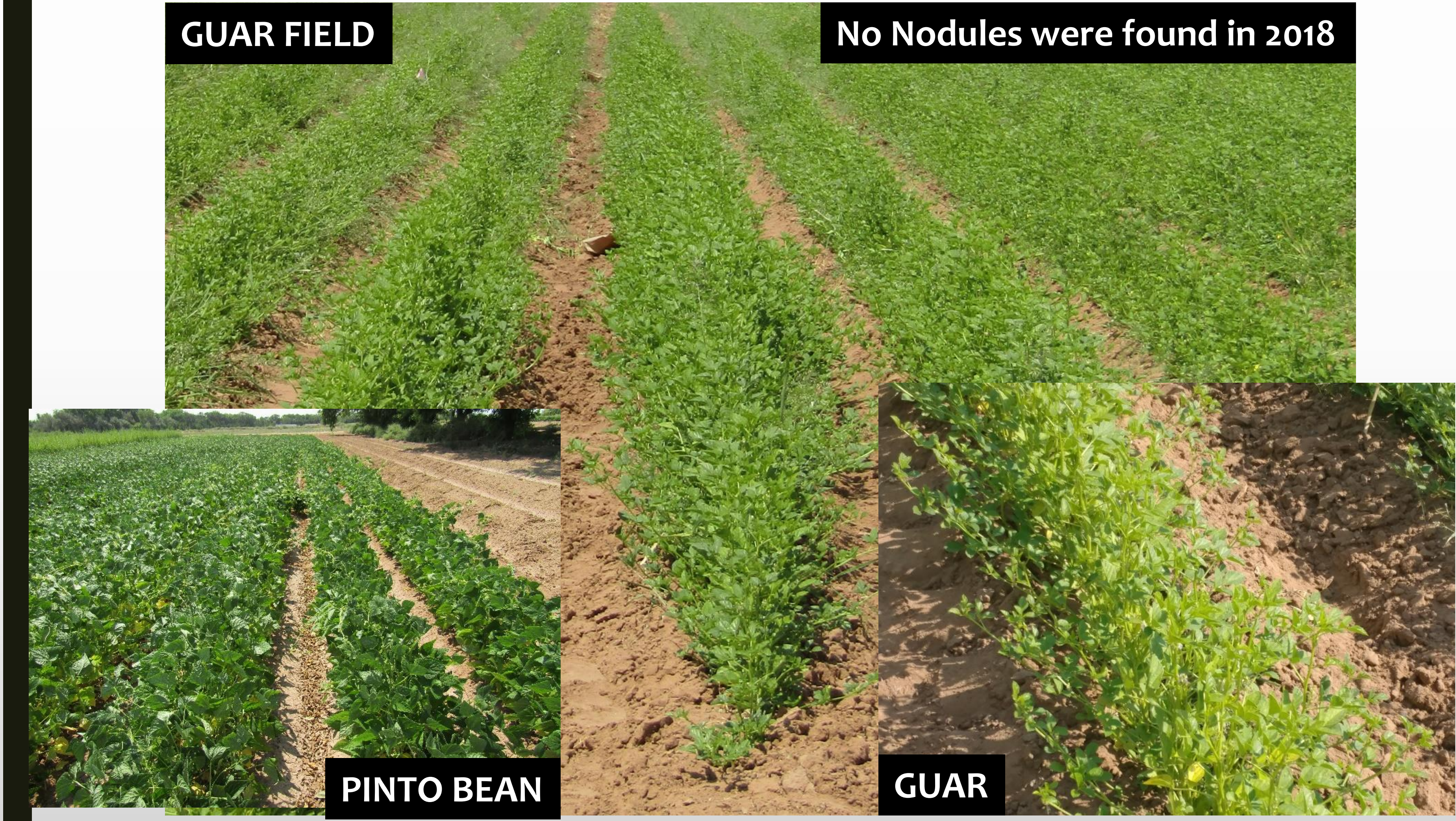
Results – Los Lunas/Clovis

Inoculum and Phosphorus Response



No Nodules were found in 2018

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Guar, Los Lunas, 2019

Impact of Nitrogen and Phosphorus on guar growth and yield

Guar Field – September 9, 2019

Nodules (1 month old Guar)

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On-Farm Trials

- Two on-farm, farmer-managed trials on guar in southern NM (Dona Ana County)
- Trial started in June 2019
- Both trials were hosted by **organic farmers**
- One of the trials was terminated due to weed pressure
- Weed control options are very limited for organic producers
- Plan to work with conventional growers next season (they have more weed control options)
- A large (11 ac) guar demonstration trial in eastern NM could not take place – farmer withdrew due to rain-induced planting delay

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Some Challenges

- Equipment adaptation for guar production
  - *Planting*
  - *Harvesting*
- Weed control in guar
  - *Legumes are slow starters*
  - *Weed infestation can be very rapid*
  - *Planning to work with a weed scientist on guar in NM*

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Equipment Adaptation

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Weed Issues

Spike of Palmer Amaranth Weed

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On-Farm Trial in La Union, NM

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United States Department of Agriculture
National Institute of Food and Agriculture

Guar, Los Lunas, 2019

Impact of Nitrogen and Phosphorus on guar growth and yield

Clean Field with Low Weed pressure

- No Manual Weeding
- Preplant Herbicide and Cultivation
- High Seeding Density

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Guayule in Las Cruces, NM

- Two on-station trials are on-going
  - *Direct seeded guayule &*
  - *cold tolerance study*

Direct Seeded Demonstration Trial

Cold Tolerance Study

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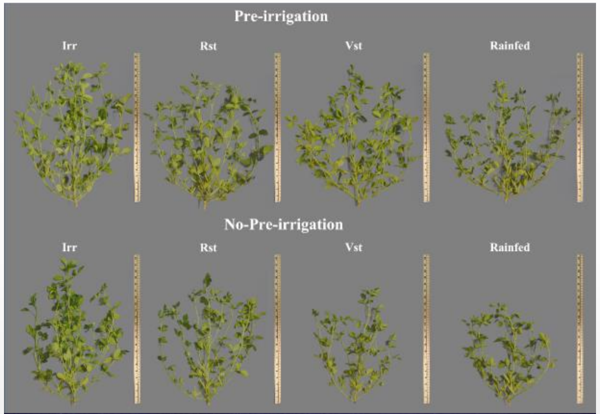
United States Department of Agriculture

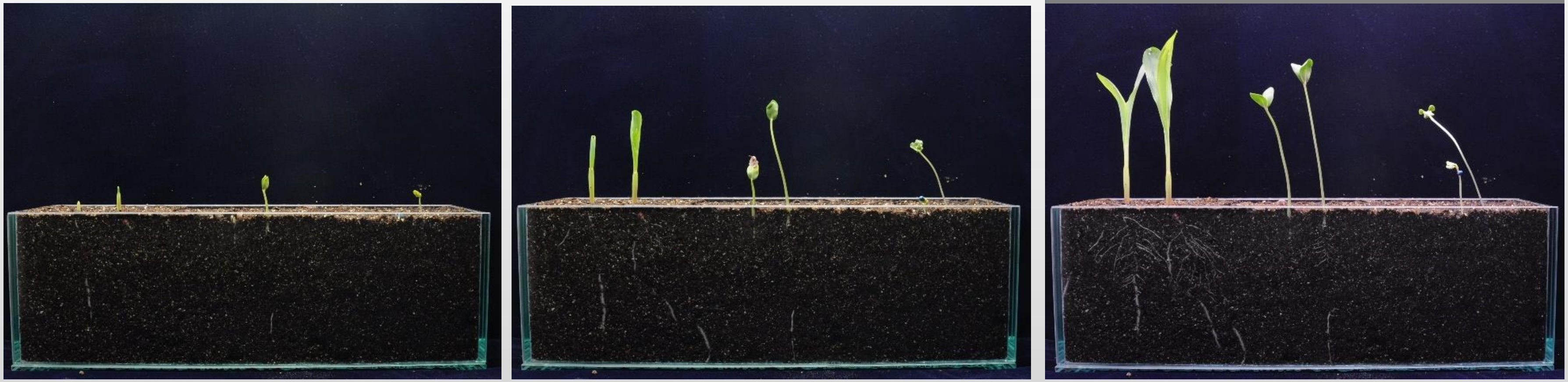
National Institute of Food and Agriculture

Task: Developing guar developmental videos from field and incubators studies for educational team.

Objective: Develop time lapse videos of guar germination, growth and development under different management.

- A few trial runs have been conducted both in incubator studies and field studies.
- Still photos need to be converted to time lapse videos.
- This project is being done by Dr. Angadi’s group





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Arizona/SBAR-4H Yr2 Summary

- **SBAR 4-H Biofuels Summer Camp, University of Arizona, June 3-7, 2019**
- **5-day dorm-based residential summer camp** with the theme of Biofuels, presented in the context of the SBAR project content and goals
  - *18 total youth participated*
  - *9* ***female*** *and 9* ***male*** *campers*
  - ***Campers ages:*** *11 (1 camper); 12 (12 campers); 13 (3 campers); 14 (2 campers)*
- **Camper Ethnicity:**
  - *9 Native American,*
  - *2 Hispanic/Latino,*
  - *2 multi-racial (white/Hispanic and white/black),*
  - *2 Asian/Pacific Islander*
  - *3 White*

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New Mexico/SBAR-4H Yr2 Summary

- Developed 3 workshops for SBAR 4-H and for afterschool enrichment programs.
- Visited the principals of Picacho, Mesa and Vista Middle schools in Las Cruces, NM, and recruited teachers to attended the Train the Trainer workshop held in July, 2019.
- Established collaborative efforts with
  - *NMSU 4-H Youth and Development Office*
  - *Dona Ana County Extension Office*
  - *Coordinator for K-12 Science Instruction in Las Cruces Public Schools.*

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New Mexico/SBAR-4-H

- 4-H Youth Get-Away 2019,
  - *Guar Giant Bubble Workshop Glorieta, NM.*
  - *A total of* ***180 junior age middle school students*** *participated in the workshop.*
  - *Students learned the science behind how bubbles work, and the effect of guar gum on bubble shape and size.*
- State 4-H Conference Workshop and Table Display.
  - *SBAR-EEO/State 4-H Conference activity for 23 high school students and table display*

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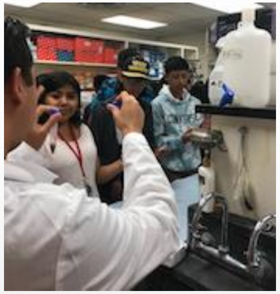
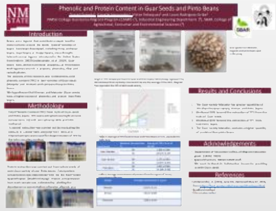
Extension, education and outreach

- **Thirty-eight Native American high school students,** and **eight NMSU freshman Hispanic** students participated in the SBAR Workshop:
  - *Antioxidant capacity of guar seeds extracts on June 19, 2019.*
- Two NMSU sophomore students from the College Assistance Migrant Program (CAMP) Participated in the 2019 medicinal plants summer
  - *presented the research poster: "Phenolic and Protein Content in Guar Seeds and Pinto Beans" on June 21, 2019.*
- Twenty seven SBAR teachers and fellows participated in the Separation of a mixture workshop on July 02, 2019.





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Plans For Year 3

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Arizona Extension

- Plans for year 3
  - *Continue to produce newsletter updates and discuss SBAR progress at field days and agriculture events.*
  - *Continue to engage Native American communities and collect feedback on guayule.*
  - *Begin to create Extension documents as field research is completed.*
  - *Increase 1-on-1 meetings with growers and ag industry professionals to better gauge interest and collect accurate feedback.*

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NM SBAR Extension- Year 3

- Information dissemination on SBAR/Guar will continue
- **On-farm** and **on-station** demonstration trials on guar will continue
- On-farm trials will focus on conventional systems
- Lesson learned from 1st and 2nd years of the project will be used to improve on-farm trials
- Field day presentations on Guar at different sites
- Interaction with growers will continue

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NM SBAR Extension- Year 3

- Guar extension publications will be written and published
- Plan to release quarterly newsletters to keep in touch with contacts
- Plan to host a conference on alternative crops in eastern NM
- Better documentation of contacts

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NM/AZ SBAR 4H-Year 3

- New Mexico
  - *Outreach at least three Native American communities in NM and share the three SBAR-4-H Workshops developed on year two.*
  - *Complete the "Alternative Crops for NM booklet" for youth materials for SBAR 4-H.*
  - *Outreach After-School programs for middle school Junior age students.*
- Arizona
  - *4-H will be more focused on encouraging activities within the AZ counties.*
  - *More 4-H efforts among the native Americans and in tribal lands.*

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EDUCATION

LEAD: *Sara Chavarria*

Team: *Anderson, Brewer, Duncan, Fields, Knox,*
Molnar, Ogden, Rogstad, Sikora

Education Team – Year 2

Sara Chavarria & Corey Knox – UA Education

Catie Brewer – NMSU Engineering

Torran Anderson & Cara Duncan – UA SBAR Coordinators

Stephanie Sikora – UA Institute for Energy Solutions

Istvan Molnar – UA Agriculture and Life Sciences

Kim Ogden – UA Engineering

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Year 2: APPROACH for K-12 SBAR Lesson Plans

DRIVEN BY: Understanding Our Arid Lands Through Case Studies

1. Arid Lands: Introductory Lessons on Environmental, Social, and Economic issues
2. Arid Lands: Plants, Soil, and Water
3. Arid Lands: Careers, Agriculture, and Industry
4. Arid Lands and Climate Change/Adaptation
5. Arid Lands and Environmental Stewardship
6. Arid Lands Other
    1. *Space Colonization*
    2. *Energy & Matter*
    3. *Future Studies Implications*

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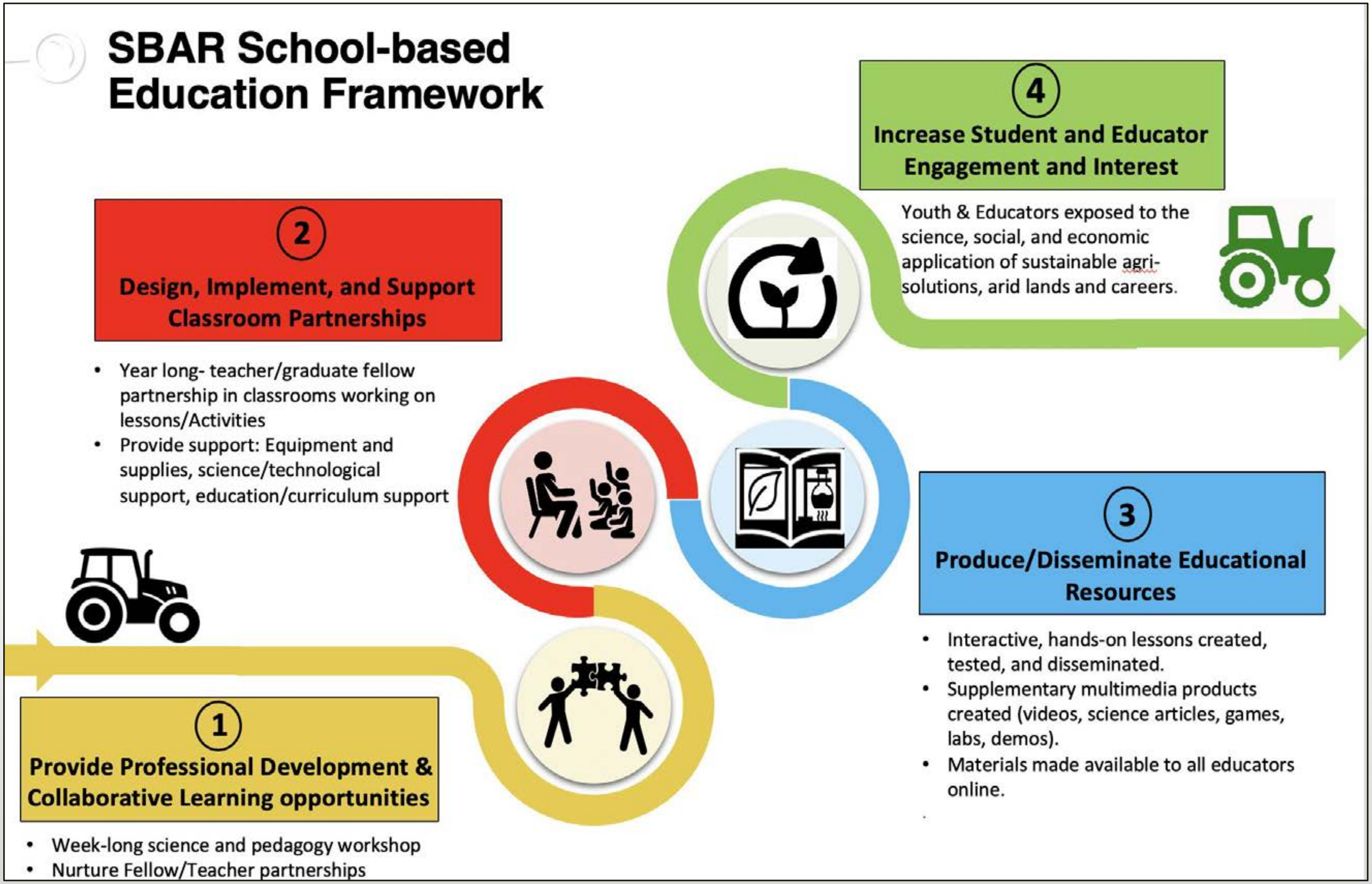
Year 2: K-12 Framework Context



SBAR School-based Education Framework

1
Provide Professional Development & Collaborative Learning opportunities

- Week-long science and pedagogy workshop
- Nurture Fellow/Teacher partnerships

2
Design, Implement, and Support Classroom Partnerships

- Year long- teacher/graduate fellow partnership in classrooms working on lessons/Activities
- Provide support: Equipment and supplies, science/technological support, education/curriculum support

3
Produce/Disseminate Educational Resources

- Interactive, hands-on lessons created, tested, and disseminated.
- Supplementary multimedia products created (videos, science articles, games, labs, demos).
- Materials made available to all educators online.

4
Increase Student and Educator Engagement and Interest

Youth & Educators exposed to the science, social, and economic application of sustainable agri-solutions, arid lands and careers.

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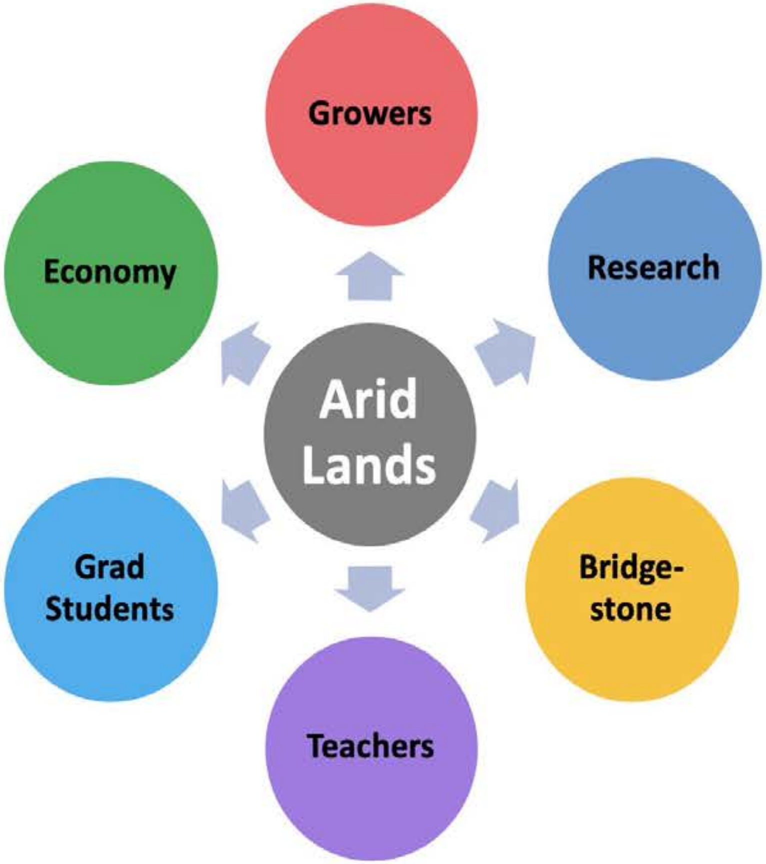
Year 2: K-12 - Making It Personal

'Understanding our Arid Lands' drives the activity design



-What will it cost me?
-How profitable is growing guayule to me?
-Why should I grow guar or guayule on my farm?
-What changes do I need to make to grow guar and guayule successfully?

-Are there arid crops that can grow with little water in the desert?
-What will these agriculture experiments teach us?

-How can we use the 90% of the crop so farmers make money?
-How can we partner with growers so they benefit from growing guar and guayule?

-How does this benefit my student's community?
-What careers for youth can we explore?

-How can I communicate my science expertise to younger audiences?
-Career questions – What career paths exist for me?

-What can we grow guar and guayule to produce - fuel, resins, rubber, other?

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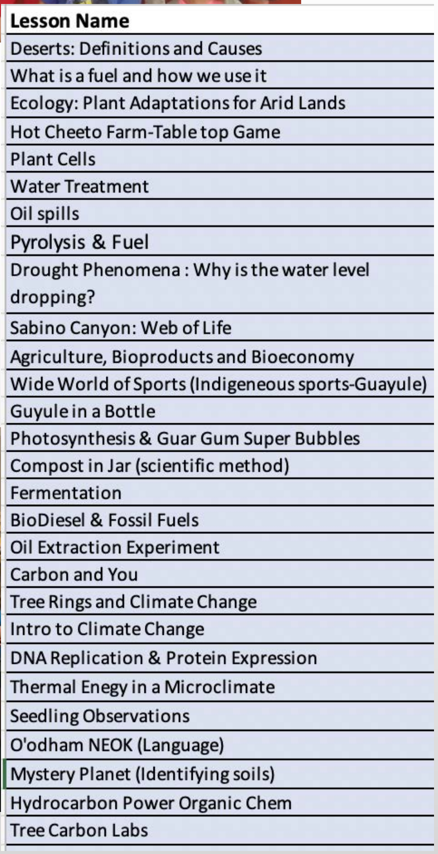
Year 2 Highlights

- Guardians of the Biosphere afterschool program (NM)
- Field trips to Bridgestone (AZ)
- Teacher/Fellow partnerships begin
  - *4 teacher/fellow teams – AZ*
  - *2 teacher/fellow teams - NM*
- Fellow seminar launched: topics addressed are lesson design, classroom engagement practices, presentation to youth, lesson delivery.
- Over 20 lessons, activities, powerpoints, videos developed by fellows and teachers
- Education team identified 5 lessons (with accompanying ppts & videos) for web posting to work on and refine.



Lesson Name
Deserts: Definitions and Causes
What is a fuel and how we use it
Ecology: Plant Adaptations for Arid Lands
Hot Cheeto Farm-Table top Game
Plant Cells
Water Treatment
Oil spills
Pyrolysis & Fuel
Drought Phenomena : Why is the water level dropping?
Sabino Canyon: Web of Life
Agriculture, Bioproducts and Bioeconomy
Wide World of Sports (Indigeneous sports-Guayule)
Guyule in a Bottle
Photosynthesis & Guar Gum Super Bubbles
Compost in Jar (scientific method)
Fermentation
BioDiesel & Fossil Fuels
Oil Extraction Experiment
Carbon and You
Tree Rings and Climate Change
Intro to Climate Change
DNA Replication & Protein Expression
Thermal Enegy in a Microclimate
Seedling Observations
O'odham NEOK (Language)
Mystery Planet (Identifying soils)
Hydrocarbon Power Organic Chem
Tree Carbon Labs

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Year 2 Highlights: Teacher/Fellow Workshop

Summer 2019 – Teacher workshop – part 1 (NM) – 2.75 days

- Immersive SBAR content exposure that included:
  - *Pre-Engineering (PREP) Middle School Summer Academy biofuel activity*
  - *Individual lectures around biochemistry topics*
  - *Tactile learning activities*
  - *Lab demonstrations*
  - *Tours*
    - Labs
    - Field sites

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Year 2 Highlights: Teacher/Fellow Workshop

Summer 2019 – Teacher workshop – part 2 (AZ) – 5 days

- Collaborative building approach to explore how to incorporate content into classrooms
  - *Mind-mapping*
  - *Reflection activities*
- Curriculum map connections - Week 1 topics reviewed to seek matches in their classrooms
- Presentations by Cohort 1 teachers on lessons they have developed
- Presentations by Extension and Arid Lands experts
- Lesson Design time for teams (Cohorts 1 & 2)
- Lesson Editing time for teams (Cohort 1)
- Educators finalize Year 2 fellow designed lessons

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Year 2 Highlights: Graduate Program

Applied Biosciences – Professional Science Master's Graduate Interdisciplinary Program

- Consent of all partner departments with classes was secured.
- Departments:
  - Biostatistics
  - Environmental Science
  - Physiology
  - Biosystems Engineering
  - Plant Sciences
  - Chemical and Environmental Engineering
  - Biochemistry
  - Molecular and Cellular Biology
  - Animal and Comparative Biological Sciences
  - Ecology and Evolutionary Biology

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SBAR

USDA United States Department of Agriculture National Institute of Food and Agriculture

YEAR 3 Targets

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Education—New Mexico Year 3

- Four teachers and fellows
- Expand into 4th and 5th grade science
- Year 2 of Guardians of the Biosphere afterschool program
- Train-the-Trainer Workshop for 4-H agents and volunteers

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Education -Arizona Year 3

Fall 2019 :

- Layout on Website & Template for Lessons (to look professional and uniform)
- Post initial set of lessons, PPTs, and resources designed by EDU team and teachers.
- Finalize teacher professional development (PD) approach for year three = Cohort 1 teacher led workshops (using their lessons) for new teachers (cohort 3).
  - *Determine if going digital or live or **both** for PD. Determine length of PD per teacher feedback.*

Spring 2020:

- Promote summer PD opportunity. Offer programming in both NM and AZ.
- Recordings of Teacher/Fellow demos and activities for use in digital PD efforts.

Summer 2020

- Deliver Teacher PD – provide stipend for participation and evaluation.
- Assist Cohort 1 teachers to design in-person PD
- Assist Cohort 2 teachers to refine their lessons

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Education – Arizona Year 3

Applied Biosciences – Professional Science Master's Graduate Interdisciplinary Program

- The proposal will route to the University of Arizona Grad Council for final approval.

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