ACCOMPLISHMENTS REPORT
Western Regional Biomass Research Center (WRBRC)
October 2015 – September 2017
Submitted by: Hussein Abdel-Haleem, Coordinator

Locations Reporting:

Albany, California (Western Regional Research Center)
- **Crop Improvement and Genetics Research Unit**
- **Bioproduct Research Unit**

Hilo, Hawaii (Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center)
- **Tropical Plant Genetic Resources and Disease Research**

Maricopa, Arizona (U.S. Arid Land Agricultural Research Center)
- **Plant Physiology and Genetics Research Unit**
- **Pest Management and Biocontrol Research**
- **Water Management and Conservation Research**

Parlier, California (San Joaquin Valley Agricultural Sciences Center)
- **Water Management Research Unit**

Riverside, California (US Salinity Laboratory)
- **Water Reuse and Remediation Research**

Research Focus:

1. **Feedstock Development**
2. **Feedstock Production**
3. **Conversion and Co-product Utilization**

(1) Feedstock Development:

A. Accomplishments:

New roles for lipid droplets in carbon/energy balance and stress response in plants. Lipid droplets are subcellular organelles that store oil, and in oilseeds, they accumulate to high levels for the storage of triacylglycerols, which serve as a carbon and energy reserve for germinating seedlings. Lipid droplets are also present, however, in all vegetative cell types, where their roles in plant biology are less understood. ARS scientists in Maricopa, Arizona, in collaboration with scientists at the University of Guelph, Ontario, Canada, and the University of North Texas, Denton, Texas, characterized a group of proteins called Lipid Droplet-Associated Proteins, or LDAPs, that are abundant proteins that coat the surface of non-seed lipid droplets. The studies revealed that LDAPs were required for regular changes in lipid droplet abundance throughout the day/night cycle, as well as rapid increases in lipid droplet abundance during heat and cold stress response. These and other findings reveal new and unexpected roles for lipid droplets in plant biology and will be of greatest interest to other scientists working to characterize the molecular mechanisms of carbon/energy balance and stress response in crop plants.

Identification of the missing link in oil production in plant cells. In the early 1960s, the enzymatic reactions of oil synthesis were characterized and shown to involve three sequential additions of fatty acids to the three positions of glycerol. Since then, genes encoding the second and third reactions have been identified, but the first step has remained elusive. In collaborative research between scientists at the University of Alberta and ARS in Maricopa, Arizona, a gene called glycerol-3-phosphate acyltransferase (GPAT) 9 in the model plant Arabidopsis that has all of the hallmarks of the missing link has been identified. The enzyme was shown at the biochemical level to catalyze the first reaction of oil synthesis, it was localized to the correct organelle in the cell, and over-expression and knockdown of the gene had positive and negative effects on oil production in plants, respectively. Taken together, these results, and additional results from a competing research group, strongly support the identification of GPAT9 as the key remaining gene for oil synthesis in plants. These findings will stimulate many additional studies on the activity and variability of the GPAT9 gene in plants, with the end goal of increasing oil content and yield in oilseed crop plants.

Production of high amounts of oil in the leaves of plants. The fatty acid components of plant oils are chemically similar to the long-chain hydrocarbons of fossil oil, and as such, represent outstanding potential sources of renewable fuels and feedstocks for industry. The demand for transport fuels and chemicals, however, is far greater than what agriculture can typically deliver. Scientists at the ARS center in Maricopa, Arizona, in collaboration with scientists at the ARS lab in New Orleans, the University of North Texas, and the University of Guelph, investigated whether it was possible to produce high amounts of oil in the vegetative biomass of plants (e.g., leaves and stems), which has far greater biomass than that of seeds, the typical source of plant oil. By using a combination of different genes and mutant backgrounds, the oil in leaves of the model oilseed plant Arabidopsis thaliana was increased over 50-fold, and in some lines, additional genes were expressed for production of high value conjugated fatty acids, which have important uses in both nutrition and industry. Together, these findings open new avenues of research for producing high amounts of biofuels or industrial chemicals in dedicated, non-food bioenergy crops and increasing the energy density of forage crops. The work is of greatest interest to other scientists focused on increasing the energy density of crop plants and developing new and improved uses of oilseeds as feedstocks for food, feed, biofuels, and bioproducts.

Expression of castor LPAT2 enhances ricinoleic acid content at the sn-2 position of triacylglycerols in lesquerella seed. Identification of a specific gene to enhance production of castor oil in lesquerella. Castor seed oil is a conventional source of valuable hydroxy fatty acid (HFA) which has numerous industrial applications. Lesquerella seed oil also contains HFA, but unlike like castor seed which contain toxin ricin, lesquerella seeds represent a safe source of HFA. If lesquerella oil can be engineered to resemble castor oil, it would provide an alternative source of castor oil that is safe, cost-competitive, and readily adaptable by existing industrial technologies. ARS scientists discovered a key castor gene as a target for genetic engineering a castor oil-producing lesquerella crop. This gene, lysophosphatidic acid acyltransferase 2 (RcLPAT2), was used to create transgenic lesquerella plants in collaboration with Washington State University at Pullman and Rothamsted Research at United Kingdom. The result is the first demonstration that RcLPAT2 can be used to increase HFA, a valuable property for the engineering of a new castor oil-producing crop, such as lesquerella, Camelina, and canola.

Characterization of leaf cuticular waxes and cutin constituents of Camelina and closely-related species. The cuticle of plants has low permeability to water, which influences dehydration avoidance and drought tolerance of crop plants. In collaboration with scientists at West Virginia University, ARS researchers in Maricopa, Arizona detected the variations in leaf wax and cutin constituents, traits related to abiotic stress tolerance, in seventeen accessions of Camelina species. Camelina species exhibited a wide range of wax and cutin contents, where primary alcohols and alkanes are the predominant classes of leaf wax. Among the cutin monomers examined, dihydroxy monobasic acids were the highest in concentration. This work lays the foundation for identifying specific monomers as potential targets for breeding efforts to improve drought tolerance in Camelina.

Characterization of the genetic diversity in Brassica napus. To expand the resources available for breeding of B. napus, ARS scientists in Maricopa, Arizona, Peoria, Illinois, Morris, Minnesota, Sidney, Montana, Mandan, North Dakota, Temple, Texas, Ames, Iowa, Akron, Colorado, Pendleton, Oregon, and scientists from Idaho State University, and Cornell University, collaborated to collect and genetically characterize a global population of Brassica napus plants. Eight hundred Brassica lines were genotyped using genotyping by sequencing (GBS) technology. Comparison of the DNA sequences revealed three distinct and diverse groups distinguished by growth habit and geographical origin including spring, winter-European and winter-Asian subgroups. Each subgroup had a different historical evolutionary path, which was also reflected in the traits and genes that each subgroup possessed. This work provides insight to the diversity of plants available within the B. napus population and also provides single nucleotide polymorphism (SNP) markers that can be used to identify genes and genomic regions that are associated with traits of interest. This work will thus be of greatest interest to those scientists interested in improving the agronomic performance of B. napus as a valuable oilseed crop used for food, feed, and biofuel purposes using genome-assisted techniques.

Genetic characterization of the US national guayule germplasm collection. Natural rubber is a critical raw material of modern society, essential to a diverse range of industries such as automotive, electronics, clothing, and health care. However, 99.9% of natural rubber is derived from a single source, the rubber tree (*Hevea brasiliensis*), and 75% of it is produced in South Eastern Asia. Guayule (*Parthenium argentatum*) is a woody perennial shrub native to the desert regions of northern Mexico and southwestern United States that produces rubber in its bark tissues, but attempts to increase rubber yields through crop breeding have been hampered by a lack of well-characterized germplasm. To help address this issue, scientists at Cornell University, West Virginia University, and the ARS lab in Maricopa, Arizona, performed a detailed assessment of all publically available guayule germplasm, including closely related species and interspecific hybrids. By using a combination of next generation sequencing technologies and phylogenetic approaches, the scientists could clearly determine the genetic identity and relationships for each accession. Overall, these data help to identify specific lines that can be used for crop breeding, identify geographical regions that should be explored to obtain additional genetic diversity, and provide robust molecular tools to enable genomics-assisted crop improvements. This study is a great resource to the guayule research community and represents a substantial step forward in the development of guayule as an alternative, commercial source of natural rubber.

Phenotypic characterization of guayule USDA collection under field conditions. Among the more than 2000 plant species that synthesize natural rubber (NR), only rubber tree (*Hevea brasiliensis* Muell. Arg.) and guayule (*Parthenium argentatum* A. Gray) are growing on commercial scale. Because of changes in NR demand, vulnerability of its prices, and the threats of rubber tree diseases and natural disasters, guayule is sustainable, domestic and alternative NR source as well resource for latex, resins, and biofuels. Guayule is a potential new crop for arid-lands and low input regions of southwestern USA. The ARS scientist is phenotyping USDA guayule collection were grown in replicated field trial at Maricopa, Arizona under full irrigation and dry conditions. Traditional morphological (plant height, canopy volume and perimeter, main branches number, stem thickness and leaf) traits and High throughput phenotyping techniques (crop height, canopy multi-spectral reflectance indexes and canopy temperatures) are measured. Results indicated the variations in growth vigor among guayule accessions under favorable growth conditions (well irrigation). The growth vigor of guayule accessions were suppressed in response to drought stress (dry conditions) compare to those grown under favorable conditions. These phenotypic variations among guayule accessions as well their variable response to the environmental stresses will lead to identifying parental candidates to be used in guayule breeding programs to increase the genetic gains of traits related to growth vigor, and consequently enhance rubber and latex yield and production.

Transgenic Guayule for enhanced Isoprenoid production. Economic sustainability for the developing guayule crop in the SW USA might be secured with increased natural rubber yield. ARS scientists in Albany, CA have successfully developed tools and techniques to engineer guayule plants with higher rubber yield. In 2017, two US patents were issued, both of which enable production of higher levels of isoprene pyrophosphate, the monomer used by plants to synthesize natural rubber polymers. U.S. Patent US 14209,255 (December 20, 2016) is a method to transform the chloroplasts of guayule. U.S. Patent 9,574,203 (February 21, 2017) covers guayule plants transformed to overexpress a critical enzyme in the isoprenoid pathway. These technologies are now available to guayule breeders and growers for developing high rubber content lines.

Engineering rubber production in guayule plants. Genetically improved guayule lines. Increasing yield of natural rubber in guayule plants is the main goal of current genetic improvement studies. ARS scientists at Albany, California, discovered a single gene modification that can increase rubber content by up to four fold in the laboratory. During 2016, greenhouse evaluations confirmed the increase in rubber content, and also discovered the same gene results in larger, greener plants. This remarkable combination could increase grower yields though both higher % rubber and higher biomass. The plants have been transferred to an industrial partner for field evaluation. CRIS Project# 2030-21410-021-00D

Jerusalem artichoke a potential biorefinery crop for underused and saline soils of the semiarid. Jerusalem artichoke (Asteraceae) is a North American crop that was consumed by Native Americans before European settlers arrived. Its tubers are a rich source of inulin (50% DW), a fructan (polymer of fructose molecules) valued as a non-caloric probiotic fiber and source of sugars for biofuels. The whole plant can provide inulin, protein (6-12%), and aminoacids as feed and food. Although reported as tolerant to drought and salinity, ARS scientists at Riverside, California found that salinity effects on the production of inulin, inulin's degree of polymerization, and tuber antioxidant capacity have not been reported. Its high adaptability to diverse edaphoclimatic conditions, pests and diseases, and ability to re-sprout from overwintered tubers make it a potential biofuel, food, and feed crop adaptable to areas unsuitable for conventional agricultural crops. This high adaptability of the crop to saline waters and its short cycle make it also a potential biofuel crop for northern USA and Canada, which have only approximately four months of the summer season.

Effective methods for doubling the switchgrass genome. Switchgrass is a high-biomass perennial grass adapted to growth in the midwestern United States. In order to improve its potential as a biofuel crop, removing genetic barriers that prevent the formation of hybrids between switchgrass subpopulations with differing numbers of chromosomes is desirable. To derive plants with twice their original number of chromosomes, ARS scientists in Albany, California, and Lincoln, Nebraska, treated young seedlings with inhibitors of cell division. This method produced fertile plants with double the genome size and new characteristics including increased seed size, which has been associated with improved seedling vigor and successful establishment after planting. Moreover, these plants can be crossed with other plants with matching chromosome numbers to bring together genes and traits from subpopulations that had not previously been compatible for breeding.

B. Citations:

Chen, G.Q., Van Erp, H., Martin-Moreno, J., Johnson, K., Morales, J.S., Browse, J., Eastmond, P.J., Lin, J.T. 2016. Expression of castor LPAT2 enhances ricinoleic acid content at the sn-2 position of triacylglycerols in lesquerella seed. International Journal of Molecular Sciences. 17(4):507. doi:10.3390/ijms17040507.

Dias, N.S., Ferreira, J.F., Liu, X., Suarez, D.L. 2016. Jerusalem artichoke (Helianthus tuberosus, L.) maintains high inulin, tuber yield, and antioxidant capacity under moderately-saline irrigation waters. Industrial Crops and Products. 94:1009-1024. doi: 10.1016/j.indcrop.2016.09.029.

Dong, N., McMahan, C., Whalen, M., Cornish, K., Coffelt, T. 2017 Trangenic Guayule for Enhanced Isoprenoid Production. US Patent #9,574,203 issued Feb. 21, 2017.

Gazave, E., Tassone, E.E., Ilut, D.C., Wingerson, M., Datema, M., Witsenboer, H., Davis, J.B., Grant, D.M., Dyer, J.M., Jenks, M.A., Brown, J., Gore, M.A. 2016. Population genomic analysis reveals differential evolutionary histories and patterns of diversity across subgenomes and subpopulations of Brassica napus L. Frontiers in Plant Science. 7:525. doi: 10.3389/fpls.2016.00525.

Gidda, S.K., Park, S., Pyc, M., Yurchenko, O., Cai, Y., Wu, P., Andrews, D.W., Chapman, K.D., Dyer, J.M., Mullen, R.T. 2016. Lipid droplet-associated proteins (LDAPs) are required for the dynamic regulation of neutral lipid compartmentation in plant cells. Plant Physiology. 170:2052-2071.

Ilut, D.C., Sanchez, P.L., Coffelt, T.A., Dyer, J.M., Jenks, M.A., Gore, M.A. 2017. A century of guayule: Comprehensive genetic characterization of the US national guayule (Parthenium argentatum A. Gray) germplasm collection. Industrial Crops and Products. 109:300-309

Kang, Byung-guk, McMahan, Colleen M., Whalen, Maureen, Dong, Niu, Engineering rubber production in plants, US Patent 9,523,097 issued Dec. 20, 2016

Lin, J.T., Fagerquist, C.K., Chen, G.Q. 2016. Ratios of regioisomers of the molecular species of triacylglycerols in lesquerella (*Physaria fendleri*) oil estimated by mass spectrometry. Journal of the American Oil Chemists' Society. 93:183-191. doi: 10.1007/s11746-015-2769-2.

Pyc, M., Cai, Y., Greer, M.S., Yurchenko, O., Chapman, K.D., Dyer, J.M., Mullen, R.T. 2017. Turning over a new leaf in lipid droplet biology. Trends in Plant Science. 22:596-609.

Singer, S.D., Chen, G., Mietkiewska, E., Tomasi, P., Jayawardhane, K., Dyer, J.M., Weselake, R.J. 2016. Arabidopsis GPAT9 contributes to synthesis of intracellular glycerolipids but not surface lipids. Journal of Experimental Botany. doi: 10.1093/jxb/erw242.

Tomasi, P., Wang, H., Lohrey, G.T., Park, S., Dyer, J.M., Jenks, M.A., Abdel-Haleem, H.A. 2017. Characterization of leaf cuticular waxes and cutin monomers of Camelina sativa and closely-related Camelina species. Industrial Crops and Products. 98:130-138. doi: 10.1016/j.indcrop.2017.01.030.

Yoon, S., Aucar, S., Hernlem, B.J., Edme, S.J., Sarath, G., Blumwald, E., Tobias, C.M. 2016. Generation of octoploid switchgrass by seedling treatment with mitotic inhibitors. BioEnergy Research. doi: 10.1007/s12155-016-9795-2.

Yurchenko, O., Shockey, J.M., Gidda, S.K., Silver, M.L., Chapman, K.D., Mullen, R.T., Dyer, J.M. 2017. Engineering the production of conjugated fatty acids in Arabidopsis thaliana leaves. Plant Biotechnology Journal. 15:1010-1023. doi: 10.111/pbi.12695.

(2) Feedstock Production

A. Accomplishments:

Agave as a bioenergy feedstock on arid lands. *Agave americana* is a high-yielding plant recognized as a potential bioenergy crop for dry regions of the world. ALARC scientists and collaborators from Ohio University completed a three-year agave irrigation experiment in Maricopa that included four annual levels of irrigation (100 mm). This is the first field study to determine if *A. americana* can be grown productively as a bioenergy crop in desert regions of the Southwestern U.S. We found that the highest agave biomass yield and water use efficiency was obtained with only 330 mm of annual irrigation. Results suggest that agave could become a bioenergy feedstock in semi-arid and arid U.S. regions and has very low irrigation water requirements compared to the traditional crops grown in the region. This research will benefit potential Agave farmers in semi-arid and arid regions, water management agencies, and the biofuel industry.

Surface irrigation management for guayule rubber production in the US desert southwest. The high demand and expected shortages of natural rubber from overseas have brought renewed interest in commercializing the desert shrub, guayule, in arid areas of US Southwest for its natural rubber. However, growing guayule in desert regions where water is limited requires prudent and efficient irrigation management. This research conducted by ARS scientists in Maricopa and expands existing but very limited information on how to manage irrigation for guayule in desert areas. Guayule growth and rubber yield responses to irrigation were studied during a 29-month experiment in Maricopa, Arizona. The study shows that maximum guayule rubber yield occurred with the most irrigation applied. However, rubber yields were only 8% lower than maximum with significantly less irrigation. This research will be useful for commercial guayule growers when considering irrigation management and water use efficiency. The research will also be of interest to the US Rubber Industry, including Tire Manufacturers, irrigation consultants, water district water managers, and other research investigators of guayule.

Lesquerella seed yield estimation using color image segmentation to track flowering dynamics under water and nitrogen limitation. Lesquerella seed oil may be used as a biorenewable petroleum substitute in the production of many industrial products, including cosmetics, coatings, plastics, and greases. It also has application as a biorenewable diesel fuel additive. Several issues related to crop management and plant breeding must be resolved before the crop can be produced commercially. ARS scientists in Maricopa, Arizona in collaboration with scientists from University of Arizona, Tucson, investigated a digital imaging approach to track the dynamics of flowering in lesquerella under different levels of irrigation and fertilizer management. It was demonstrated that digital images were useful to detect changing flowering patterns and that flowering patterns were highly correlated with lesquerella seed yield. Information about lesquerella flowering can aid breeders in the selection of optimum varieties and can aid producers with irrigation management and harvest decisions. The results advance the science of digital image processing for applications in agricultural crop management. Results will benefit plant breeders, growers, and others aiming to develop lesquerella into a commercially viable oilseed crop for production of biorenewable products.

<u>Host plant preference of Lygus hesperus exposed to three desert-adapted industrial crops.</u> It is well known that the lygus bug feeds on a wide variety of plants (i.e., cotton, strawberry, weeds, etc.). It is also known that this pest exhibits a wide host plant feeding preference for the various types of plants. The desert-adapted crops vernonia, lesquerella, and camelina are being grown in the arid southwest as potential feedstock for biofuel and/or other environmentally friendly products. However, their commercial development and integration into the existing agro-ecosystem, as either replacement for conventional crops (i.e., cotton or alfalfa) or as additions to a producer's cropping system, will undoubtedly influence the structure, dynamics, and function of the existing arthropod community. Of particular interest is the potential impact of lygus on these new crops and the changes in arthropod community dynamics that may exacerbate or ameliorate pest problems in current conventional crops grown in the region. In collaboration between ARS scientists in Maricopa, Arizona and scientist from University of Illinois, Urbana, Illinois, a feeding choice test was conducted to determine the relative attractiveness of these three crops to lygus. The study revealed that lygus readily fed and laid eggs on all three plant species. However, they were observed most often feeding and laying eggs on vernonia and least often on camelina. Such basic information is essential for the successful stewardship of these crops into commercial production.

<u>Evaluating Oilseed Biofuel Production Feasibility in California's San Joaquin Valley Using Geophysical and Remote Sensing Techniques.</u> For strategic reasons alternative fuels, such as biofuel, will foreseeably continue to be a part of a planned US military goal that encompasses a wide and varied range of fuel sources in spite of their high cost. Though more costly than petroleum-based fuels and envisioned as a minor component of the overall military fuel sources, biofuels are nonetheless strategically valuable to the military because of the intentional reliance on multiple, reliable, secure fuel sources. Significant reduction in oilseed biofuel cost occurs when grown on marginally productive saline-sodic soils, which are plentiful in California's San Joaquin Valley (SJV), particularly on the west side. The ARS scientists at Riverside and Parlier, California, Fort Collins, Colorado in collaborations with scientists at University of New Mexico, Albuquerque, New Mexico and Riverside Public Utilities, Riverside, California, formulated a crop yield model relating a mustard oilseed variety yield to edaphic properties for evaluating the feasibility of oilseed production on marginal soils in the SJV to support a 115 Million Liter per year (ML yr^{-1}) biofuel conversion facility. The mustard oilseed yield model indicates that yield in the SJV is affected by boron toxicity, salinity, leaching fraction, and gravimetric water content at field capacity. Monte Carlo simulations for the entire SJV fit a shifted gamma probability density function. This shifted gamma cumulative density function indicates that there is a 0.15-0.17 probability of meeting the target biofuel-production level of 115 ML yr^{-1}. Consequently, adequate biofuel from mustard oilseed grown on salt-affected soils for the entire SJV to support a conversion plant of sufficient capacity to justify construction is highly unlikely.

<u>Beneficial soil microbe isolated.</u> Phosphorous is an important crop nutrient that can become chemically fixed in the soil making it unavailable to plants. ARS scientists in Albany, California, isolated a strain of bacteria found naturally in soil that can release chemically bound phosphorous and make it available for plant growth. Greenhouse studies showed that plants inoculated with the bacteria had much better growth compared to other plants that were grown without the bacteria in soils containing fixed phosphorous. The use of beneficial soil microbes could reduce the amount of phosphorous fertilizers needed for crops and reduce the problem of fertilizer runoff from fields into waterways. CRIS Project #2030-41000-058-00D

Mini-pilot scale production of biofuel and animal feed from heterotrophic algae. Microalgae derived oils have outstanding potential for use in biodiesel production. *Chlorella protothecoides* has been shown to accumulate lipids up to 60% of its cellular dry weight with glucose supplementation under heterotrophic growth conditions. To reduce production costs, alternative carbon feedstocks have been evaluated and show promise as low-cost alternatives. ARS Scientists at Hilo, Hawaii determined that *C. protothecoides* isolates are capable of robust cell growth and oil production in growth medium comprised of pH-adjusted puree of culled, waste papaya fruit without any additional growth supplements. Optimization of culture medium and growth conditions were used for lab-scale strain characterization and demonstration of the potential for scale-up to an industrial bioprocess. A zero-waste approach makes agriculture more profitable and addresses food and energy security issues in Hawaii. Research continued to optimize/increase production scale of *C. protothecoides* for oil and animal feed production using papaya as a feedstock. A 40 gallon SIP Bioreactor was installed at Pacific Basin Agricultural Research Center, Hilo, Hawaii (PBARC) and results from lab scale have been successfully replicated. Demonstration phase production (>100 gal) was initiated as a collaboration between DKI-PBARC, HDOA-Agribusiness Development Corporation and Big Island Biodiesel (BIB). BIB staff were trained and SOPs developed at PBARC for algae cultures using papaya media were provided. The scale-up of the demo phase has resulted in multiple, successful 150 gallon, 7 day runs of *C. protothecoides* and papaya in 270 gallon reactors; results were comparable to small scale experiments done at PBARC.

Guayule resistance of guayule to salinity and boron on germination and seedling growth stages. Guayule has been developed and considered as an alternative crop for arid and semiarid areas of the southwestern United States, north central Mexico, and even the west side of Central California. One drawback in establishing guayule is that there are often times problems with seed germination when it is direct seeded under different soil quality conditions. If this plant is to be considered as an alternative crop for the west side of California, it must be able to grow in poor quality soils. ARS scientist at s at Parlier, California showed that an increased salinity inhibited both germination and seedling growth of six guayule lines tested. In contrast, boron positively influenced germination and growth of specific guayule lines (AZ-1, AZ-2, and AZ-4) and had no significant negative influence on the other three lines. The combination of boron and salinity, saline B-laden, increased both germination percentages and rates and seedling vigor of some lines. AZ-4 showed even a greater germination percentage in saline B-laden soil than in control soil. AZ-1 and AZ-6 showed greater germination percentages and rates than the other lines, although greater mortality percentage was observed when these AZ-1 and AZ-6 seedlings were transplanted into saline B-laden soils. These results from both growth chamber and greenhouse experiments indicate that many of the tested guayule lines can successfully germinate in typical but moderate saline B-laden soils present in the West side of central California. Consequently, guayule may be a new crop to consider for planting in this region of California.

B. Citations:

Thorp, K.R., Wang, G., Badaruddin, M., Bronson, K.F. 2016. Lesquerella seed yield estimation using color image segmentation to track flowering dynamics under water and nitrogen limitation. Industrial Crops and Products. 86:186-195.

Corwin, D. L., Yemoto, K., Clary, W., Banuelos, G., Skaggs, T. H., Lesch, S. M., & Scudiero, E. 2017. Evaluating Oilseed Biofuel Production Feasibility in California's San Joaquin Valley Using Geophysical and Remote Sensing Techniques. Sensors. 17:2343- 2368. doi.org/10.3390/s17102343

Hagler, J.R., Tassone, E.E., Coffelt, T., Lipka, A.E. 2016. Host plant preference of Lygus hesperus exposed to three desert-adapted industrial crops. Industrial Crops and Products. 89:363-367. doi: 10.1016/j.indcrop.2016.05.041.

Hunsaker, D.J., Elshikha, D.E. 2017. Surface irrigation management for guayule rubber production in the US desert southwest. Agricultural Water Management. 185:43-57.

Sarah, D.C., Kuzmick, E.R., Niechayev, N., Hunsaker, D.J. 2017. Productivity and water use efficiency of Agave americana in the first field trial as bioenergy feedstock on arid lands. Global Change Biology Bioenergy. 9:314-325.

Zhu, H., Banuelos, G.S. 2016. Influence of salinity and boron on germination, seedling growth and transplanting mortality of guayule: a combined growth chamber and greenhouse study. Industrial Crops and Products. 92:236-243. doi: 10.1016/j.indcrop.2016.07.027.

(3) Conversion and Co-Product Utilization

A. Accomplishments:

Biofuel production from Jerusalem artichoke tuber inulins. The demand for both food and fossil fuels will continue to increase in the near future due to the steady increase in world's population, while renewable sources of energy often conflict with their use also as staple crops. Thus, it is important to explore the use of biofuel crops that neither play a double role as staple crops nor compete for agricultural lands to be used for food production. Jerusalem artichoke can grow in marginal lands, saline soils, and use degraded or recycled waters to produce tubers that are rich in inulin, a fructan polymer. These inulins can be easily broken down into fructose and glucose for conversion into ethanol by fermentation. This review focuses on both tuber and inulin yields by Jerusalem artichoke, the effects of cultivar and environment on tuber yield, and on approaches to fermentation for ethanol production. Consolidated bioprocessing with K. marxianus has been the most popular approach and holds the most promise for fermentation of inulins into ethanol. Apart from ethanol, fructose can be dehydrated into to 5-hydroxymethylfurfural followed by catalytic conversion into hydrocarbons that can be used for the production of biofuels and other chemicals currently produced from petroleum such as polyesters and polyurethanes. Findings from several studies indicate that tubers alone can produce ethanol at yields that rival corn and sugarcane ethanol. Jerusalem artichoke has a tremendous potential as a bioenergy feedstock that can generate income for farmers while using recycled waters with salinity levels unsuitable for food crops.

Synergistic combinations of natural antibiotics. The use of antibiotics, which are routinely fed to livestock, poultry, and fish to promote higher yields under unsanitary conditions, is being heavily scrutinized because their persistence in the environment likely plays a role in creating antibiotic-resistant bacteria. ARS researchers in Albany, California, have developed a strategy to overcome the negative impact of residual antibiotics by creating synergistic arrays of compounds that exhibit greater antimicrobial efficacy when formulated together. For example, two amino acid type molecules that exhibit minimal antimicrobial activity when they are alone can be formulated together at high concentration, exhibiting more than a 1000-fold increase in antimicrobial activity, rivaling the efficacy of commercial antibiotics. Yet, when antimicrobial activity is no longer needed it is alleviated by lowering the concentration via dilution. The combined system falls apart into two relatively benign agents that are no more active than a typical amino acid and will thus not be a threat to promote antibiotic-resistant microbes.

A multi-enzyme scaffolding system to convert crop residues to green chemicals. Crop residues such as straw and bagasse (excess plant remaining after a product has been extracted) represent a potentially large feedstock to supply the world's fuel and chemical needs; however, for biochemical conversion, multiple different enzymes need to work together to convert complex sugars into commercially viable products. ARS researchers in Albany, California, created a way for enzymes to work synergistically by mounting them on large, multi-enzyme complexes. An artificial enzyme scaffold, a Rosettazyme that tethers up to eighteen different active enzymes onto a single platform was developed and these Rosettazymes were utilized to convert lignocellulosic material into value-added products. In one example, multiple enzymes were used to release sugars from the lignocellulosic component found in most crops. Several more tethered enzymes were employed to further convert the released sugars into their corresponding acids, called aldaric acids, which can be used as building blocks for nylon plastics. Four different types of enzymes were activated onto the same enzyme scaffold to highlight its synergy, demonstrating that tethering of multiple enzymes in a complex resulted in 71 percent more activity than using the same amount of enzymes free in solution.

Environmentally friendly "starter" charcoal. Millions of consumers use lighter fluid to light their charcoal for cooking, thereby contributing to the level of volatile organic compounds in the air around residential neighborhoods. ARS scientists in Albany, California, developed a porous charcoal material that can be easily lit without lighter fluid. The "starter" briquettes can be used to ignite traditional briquettes without the use of lighter fluid. This patented technology will help consumers comply with regional air districts' recommendations to reduce air polluting activities when air alerts are issued.

Wheat gluten-based foams as replacements for petroleum-derived foams. Most foams used in packaging and manufacturing applications are produced from petroleum derived sources and are not biodegradable. Consequently, they accumulate in landfills and can be hazardous to marine life. ARS researchers at Albany, California, have developed a high temperature process to quickly produce biodegradable wheat gluten-based foams with comparable material properties to synthetic foams. Some additives, such as cellulose fibers, minerals, zein, and other biodegradable polymers were incorporated into the foams to vary their material properties. These gluten foams can then serve as an environmentally friendly alternative to synthetic foams in packaging, manufacturing, and building applications.

Guayule research USDA-BRDI consortium. Guayule is under development in the southwestern USA as a source of domestic natural rubber, organic resins, and biofuel feedstock. Tires use almost 80% of imported natural rubber, so an understanding of the technical fit of guayule rubber for use in modern tires is critical to developing that market. In FY17, research under a consortium project funded by the Biomass Research and Development Initiative reached a successful conclusion when passenger tires built with 100% guayule rubber, in place of imported and petroleum-based rubber, passed Department of Transportation specified testing. Consortium members included ARS researchers at Albany, CA and Maricopa AZ, university partners, and rubber and tire industry leaders. Tire industry representatives also reported that the tires passed more stringent internal testing, and that a 75% guayule rubber version was suitable for sale immediately, pending material availability. A modern day technical benchmark for the use of guayule natural rubber has now been established for a commodity application.

B. Citations:

Bhagia, S., Akinosho, H., Ferreira, J.F., Ragauskas, A.J. 2017. Biofuel production from Jerusalem artichoke tuber inulins. Biofuel Research Journal. 4(1):587-599. doi: 10.18331/BRJ2017.4.2.4.

Buckley, H.L., Hart-Cooper, W.M., Kim, J.H., Faulkner, D.N., Cheng, L.W., Chan, K.L., Vulpe, C.D., Orts, W.J., Amrose, S.E., Mulvihill, M.J. 2017. Design and testing of safer, more effective preservatives for consumer products. ACS Sustainable Chemistry & Engineering. 5(5):4320-4331. doi: 10.1021/acssuschemeng.7b00374.

Jordan, D.B., Lee, C.C., Wagschal, K., Braker, J.D. 2013. Activation of a GH43 ß-xylosidase by divalent metal cations: Slow binding of divalent metal and high substrate specificity. Archives of Biochemistry and Biophysics. 533:79-87.

Jordan, D.B., Braker, J.D., Wagschal, K., Lee, C.C., Chan, V.J., Dubrovska, I., Anderson, S., Wawrzak, Z. 2015. X-ray crystal structure of divalent metal-activated ß-xyloisdase, RS223BX. Applied Biochemistry and Biotechnology. 177:637-648. doi: 10.1007/s12010-015-1767-z.

Lee, C.C., Kibblewhite, R.E., Paavola, C., Orts, W.J., Wagschal, K.C. 2017. Production of D-xylonic acid from hemicellulose using artificial enzyme complexes. Journal of Microbiology and Biotechnology. 27(1):77–83 doi: 10.4014/jmb.1606.06041.

Lhamo, D. and McMahan, C., 2017 Effect of protein addition in properties of guayule natural rubber. Rubber Chemistry and Technology. https://doi.org/10.5254/rct.17.83746

Majeed, T., Tabassum, R., Orts, W.J., Lee, C.C. 2013. Expression and characterization of Coprothermobacter proteolyticus alkaline serine protease. The Scientific World. doi: 10.1155/2013/396156.

McMahan, C. and Lhamo, D. 2015 Study of amino acid modifiers in guayule natural rubber. Rubber Chemistry and Technology 88, 2, 310-323.

Palma-Rodriguez, H.M., Berrios, J.D., Glenn, G.M., Salgado-Delgado, R., Aparicio-Saguilian, A., Rodriguez-Hernandez, A., Vargas-Torres, A. 2015. Effect of the storage conditions on mechanical properties and microstructure of biodegradabel baked starch foams. CyTA - Journal of Food. 14(3):415-422. doi: 10.1080/19476337.2015.1117142.

Rasutis, D., Soratana, K., McMahan, C., and Landis, A. 2015 A sustainability review of domestic rubber from the guayule plant. Industrial Crops and Products 70, 383-394.

Singh, S.K., Heng, C., Braker, J.D., Chan, V.J., Lee, C.C., Jordan, D.B., Yuan, L., Wagschal, K.C. 2013. Directed evolution of GH43 ß-xylosidase XylBH43 thermal stability and L186 saturation. Journal of Industrial Microbiology and Biotechnology. 41(3):489-498. doi: 10.1007/s10295-013-1377-0.

Wagschal, K.C., Jordan, D.B., Lee, C.C., Younger, A.R., Braker, J.D., Chan, V.J. 2014. Biochemical characterization of uronate dehydrogenases from three Pseudomonads, Chromohalobacter salixigens, and Polaromonas naphthalenivorans. Enzyme and Microbial Technology. 69:62-68.