# TECHNOLOGY TRANSFER REPORT



**MAY 2021** 





**Agricultural Research Service** 

# 3.0 Agricultural Research Service (ARS)

### 3.1. Mission Statement

ARS delivers scientific solutions to national and global agricultural challenges.

## 3.2. Nature and Structure of Research Program

ARS is the largest intramural scientific research agency of the U.S. Department of Agriculture (USDA). Agency goals are to find solutions to agricultural problems that affect Americans every day, from field to table, such as (a) protecting crops and livestock from pests and diseases, (b) improving the quality and safety of agricultural products, (c) determining the best nutrition for people from infancy to old age, (d) sustaining our soil and other natural resources, (e) ensuring profitability for farmers and processors, (f) keeping costs down for consumers, and (g) supporting the growth and development of rural America.

In fiscal year (FY) 2020, ARS employed approximately 2,000 scientists and postdoctoral researchers, and approximately 6,000 other employees to conduct 690 research projects at more than 90 locations. Research projects were organized within 1 of 15 national programs (see table). The Office of National Programs (ONP) in Beltsville, Maryland, plans the scope and objectives of the Agency's research projects, and five area directors implement research projects at the locations in their geographic areas.

ARS research program management, showing 16 national programs

Animal Production and Protection	Natural Resources and Sustainable Agricultural Systems	Crop Production and Protection	Nutrition, Food Safety, and Quality
Food Animal Production	Water Availability and Watershed Management	Plant Genetic Resources, Genomics and Genetic Improvement	Human Nutrition
Animal Health	Soil and Air	Crop Production	Food Safety (animal and plant products)
Veterinary, Medical, and Urban Entomology	Grass, Forage, and Rangeland Agroecosystems	Plant Diseases	Quality and Utilization of Agricultural Products
Aquaculture	Sustainable Agricultural Systems Research	Crop Protection and Quarantine	

ARS conducts a series of reviews designed to ensure the relevance and quality of its research work and maintain the highest possible standards for its scientists. Customer input helps keep the research focused on the needs of the American food and agricultural system. Plans for each active research project undergo a thorough, independent external prospective peer review managed by the Office of Scientific Quality Review. All ARS employees, including the scientific workforce, are subject to annual performance reviews, and all research scientists and engineers have technology transfer as a performance element in their annual performance appraisal. Research scientists undergo a rigorous peer review Research Position Evaluation System on a 3- to 5-year cycle. These processes ensure the continuing high-quality output of the ARS research addressing the needs of U.S. agriculture.

## 3.3. ARS Approach and Plans for Conducting Technology Transfer

Because of the delegations of authority by the Secretary of Agriculture, the ARS Office of Technology Transfer (OTT) is assigned the responsibility for obtaining patent protection for intellectual property (IP), developing strategic partnerships with outside organizations, licensing USDA technologies to the private sector and academia, and performing other activities that effectively transfer ARS research

outcomes and technologies to the marketplace. USDA's Office of the General Counsel provides legal guidance to OTT on IP matters as needed.

The ARS technology transfer program has centralized policy and approval procedures that are managed by OTT. Research agreement negotiation and implementation is decentralized and managed by the ARS area offices. Area office technology transfer staff members serve as liaisons with scientists, ARS managers, OTT, university partners, and the private sector.

To facilitate technology transfer, OTT is organized into three sections. The Partnership and Administration Section conducts day-to-day operations, coordinates technology transfer policy development, interacts with ONP on agreement policy and review, and coordinates the activities between the partnership, patenting, and licensing sections. This section maintains strong stakeholder relationships at local, regional, and national levels, ensuring the adoption of research results. This section is also responsible for coordinating, managing, and reviewing agreements, and overseeing and managing the Agricultural Research Partnerships (ARP) Network. The Patent Section of OTT provides strategic guidance to scientists regarding patent protection for their research results. The section is also responsible for receiving invention reports; convening three national patent committees (Mechanical and Measurement, Life Sciences, and Chemistry), and a Plant Protection Committee; preparing and prosecuting patent applications; and reviewing patent legal work performed by a cooperator and an ARS contract law firm. The Licensing Section of OTT manages invention licensing from all the intramural scientists in every USDA agency, including the review of license applications, negotiation of licenses, and monitoring of license agreements to assure compliance. This section also collects and disburses license revenues, manages international patent filings, and provides expert advice on all matters related to USDA invention licensing.

At ARS, technology transfer is accomplished through many mechanisms, such as:

- Developing written information for customers and stakeholders, including scientific publications, publications in trade journals, and reports to stakeholders;
- Releasing plant germplasm to the public;
- Transferring research materials to scientists outside of ARS;
- Entering into formal partnership agreements, such as Cooperative Research and Development Agreements (CRADAs) and other cooperative agreements;
- Licensing IP (patents, Plant Variety Protection certificates, and biological materials); and
- Participating in meetings with industry organizations and universities; workshops and field days;
   and distributing information to the public via the ARS Office of Communications, the National Agricultural Library, and other sources.

Because the ARS mission is to transfer technologies for broad public use by the most effective mechanism, ARS pursues patents and licensing principally to incentivize commercialization and to facilitate technology transfer to the marketplace. This is usually the case when complementary investment by the private sector is necessary to commercialize a product and patent protection is required to protect this investment. By ARS policy, patents are not filed on inventions that are considered to be only research tools. The purpose of this policy is to encourage scientific research. Judicious use of intellectual property rights (IPR) is an important cornerstone of the patent committees. IPR is used as an incentive for commercialization and full realization of the research impact of USDA technologies. In licensing practices, ARS continues to reserve the right to allow use of any IP-protected technology for research purposes (non-commercial).

OTT devised and enhanced a two-way communication mechanism between technology transfer

professionals (both at OTT and area offices), ONP, and scientists in the field through the use of technology transfer strategy calls after each patent decision and each Innovation Fund round. In FY 2020, more than 140 strategy sessions were conducted to devise customized technology transfer strategies to ensure adoption of research outcomes of each project. This was featured in the Best Practice Spotlight of Technology Transfer Tactics, a monthly newsletter, in March 2018.

Meaningful performance metrics in technology transfer are often difficult to formulate. ARS has defined better metrics for technology transfer within USDA. For example, successful outcomes for ARS may include improved agricultural practices, gathering and compilation of scientific information that enhances U.S. competitiveness, increased awareness about pathogens to help prevent human and animal diseases, or findings that help corporations and universities make informed decisions in allocating their research resources. Many of these outcomes do not require patenting or subsequent licensing for implementation.

Licensing policies also promote small business success with reasonable licensing fees in the early years and annual maintenance fees and royalties that escalate in subsequent years, sometimes after the first commercial sale of the product. Licensing further enhances commercialization by encouraging the broadest utilization of a Federal invention. ARS also incentivizes scientists on the reporting of inventions, patenting, and licensing by providing 25 percent of the license revenues to inventors (this is higher than the 15 percent required by statute). Thus, policies are in place that incentivize commercialization, minimize transaction costs, and provide fair and equitable compensation for those who create Federal innovations.

OTT founded the Agricultural Research Partnership (ARP) Network to expand the impact of ARS research by enhancing the likelihood that these outcomes will be adopted. Although replete with

ARS commercial partners with business mentoring, marketing, manufacturing, and fiscal resources needed for the success of their businesses. Consequently, the ARP Network was established to provide these complementary assets. By combining ARS research expertise with complementary capabilities and talents of partnering organizations, the ARP Network stimulates economic growth through technological advancements.

OTT collaborated with USDA's National Institute for Food and Agriculture (NIFA) to create the Small Business Innovation Research -Technology Transfer (SBIR-TT) Program. SBIR-TT encourages small businesses to collaborate with ARS researchers and/or license ARS technologies and submit an SBIR application. The relevant language in the SBIR Request for Applications states, "Additional factors that will be considered in the review process include whether an application involves a CRADA with a USDA laboratory, or a license to a USDA technology."

### 3.4. Technology Transfer Highlights

• In FY 2020, there were 107 active CRADAs, 28 of which were newly executed. The 28 new CRADAs contributed \$1,242,717 directly to ARS research projects, and approximately 68 percent of them are with small businesses. There were 323 active Material Transfer Research Agreements (MTRAs), 106 of which were newly executed. The 106 new MTRAs contributed \$1,575,241 directly to ARS research projects (see Table 1 in Section 3.5 and Figures 1 and 2 in Section 3.9).

- In FY 2020, 188 new invention disclosures were received; 76 patent applications were filed; and 40 utility patents, 3 plant patents, and 7 Plant Variety Protection Certificates were obtained. Although the year in which a patent is issued is not typically the year in which the patent application is filed, over time the ratio of patents issued over the number of patent applications filed is an indicator of "judicious" patenting. Over the last 5 years, this indicator suggests that approximately 65 percent of the patent applications result in an issued patent (see Table 2 in Section 3.5 and Figures 3, 4, and 5 in Section 3.9).
- In FY 2020, 93 new licenses were executed (80 percent with small businesses and 7 percent with universities). The total number of active licenses has steadily increased over the last 5 years from 419 to 565. Fifty-six percent of the income-bearing licenses were granted exclusively. The total income from all active licenses was more than \$3.24 million. Although the year that a license is signed is not typically the year the patent is issued, over time the ratio of newly signed licenses over the number of newly issued patents is an indicator of "judicious" patenting, considering the commercial viability of the technology and other factors. Over the last four years, this indicator suggests that approximately 45 percent of the issued patents have been licensed (see Tables 3 and 4 in Section 3.5 and Figures 6, 7, 8, and 9 in Section 3.9).
- OTT reviewed and executed licenses for the Animal and Plant Health Inspection Service (APHIS) and U.S. Forest Service.
- OTT worked with Federal Laboratory Consortium (FLC), NIFA, the National Institute of Health,
   U.S. Department of Energy, National Aeronautics and Space Administration, Small Business
   Administration, Center for Innovative Food Technology, and the University of Maryland Smith

School of Business to design strategies to enhance ARS technology transfer and economic development.

- OTT redesigned its website with new pages and content. The new website is more user friendly
  and should attract more businesses and other entities to work with ARS.
- OTT held in-person technology transfer training with 53 scientists at 6 locations in 5 States. The
   COVID-19 pandemic prevented additional in-person training, which required OTT to develop a
   virtual form of the training. More than 900 people attend the first virtual training webinar.
- OTT worked with the USDA Media and Broadcast Center to create a video explaining technology transfer and highlight ARS technology transfer successes to replace static technology transfer display cases.
- OTT worked with FLC on the Airport/Mass Transit addition to LabTech in Your Life. LabTech in Your Life- House is a virtual environment where visitors can explore the familiar setting of a home and discover successfully commercialized Federal technologies that are now commonly used household items. ARS developed 17 technologies used throughout the house. LabTech in Your Life- Airport/Mass Transit will highlight five outcomes of ARS research: Hydrangea cultivars, turfgrass cultivars, fruit and vegetable snacks, fruit and vegetable sandwich wraps, and 100-percent fruit bars.
- OTT employees served as moderators/speakers/trainers in broad technology transfer activities and forums, including the FLC national and regional meetings.
- OTT received 99 Innovation Fund applications, of which 37 were funded. The Innovation Fund, from licensing revenue, provides ARS scientists up to \$25,000 on a competitive basis for a given project per year. The purpose of the fund is move ARS research outcomes closer to adoption by

industry, academia, and other stakeholders. The first 50 projects have resulted in 5 patent applications and 2 Plant Variety Protection applications; 10 licenses; 12 publications and 1 factsheet; 4 workshops and 1 public website; and 23 university and industry collaborations.

- OTT created a LinkedIn group for the ARP Network. Through FY 2020, membership grew from 250 to more than 530. In FY 2020, there were 32 LinkedIn posts by OTT. Posts included highlighting a research topic area with an overview of the past and present ARS research program in that area, followed by a posting of technologies available for licensing in that area. In addition, other noteworthy items were posted, such as ARS news, research partnership opportunities, USDA videos on various research projects, and Federal business resources. Most posts received more than 300 views.
- OTT served on the Inter-Agency Working Group on Technology Transfer, led the Return on Investment Group on Private Sector Engagement, and represented USDA on the Lab to Market subcommittee of the National Science and Technology Council's Committee on Technology.

  OTT took the lead on the Metrics Strategic Group to establish meaningful ways in which to quantify the outcomes of Federal research and development enterprise.OTT published two peerreviewed papers: 1) M. Bahar and R. J. Griesbach, 2020. Cultivating and Nurturing A Culture of Innovation in Federal Agencies. *les Nouvelles*, September 2020, pg. 185-190, and 2) M. Bahar and R. J. Griesbach, 2020. Agricultural Extension: The Precursor to Today's Technology Transfer. *les Nouvelles*, September 2020, pg. 232-236.

## 3.5. Metric Tables

TABLE 1. Collaborative relationships for research and development							
	FY 2016	FY 2017	FY 2018	FY 2019	FY 2020		
<b>Total number of active CRADAs</b>	219	249	189	193	147		
Active CRADAs with small							
businesses	70	62	73	120	64		
Total number of newly executed							
CRADAs	39	57	51	56	28		
Newly executed CRADAs with							
small businesses	12	17	31	38	19		
Total funds to be received	\$2,113,880	\$2,804,160	\$3,121,739	\$3,280,189	\$1,242,717		
Total number of active MTRAs <sup>2</sup>	288	432	354	292	323		
Active MTRAs with small							
businesses	ND	ND	ND	ND	ND		
Total number of newly executed							
MTRAs	89	101	118	100	106		
Newly executed MTRAs with small							
businesses	ND	ND	ND	ND	ND		
Total funds to be received	\$373,953	\$743,603	\$2,267,8864	\$637,636	\$1,575.241		
Total number of active other							
agreements <sup>3</sup>	3,230	4,108	3,215	1,888	1,987		
Newly executed other agreements	756	876	621	951	1,210		
Number of newly executed MTAs	823	664	645	614	423		
Newly executed outgoing MTAs	539	445	476	398	266		
Total number of publications <sup>4</sup>							
Peer-reviewed scientific journal	4,473	4,467	4,138	3,816	3,933		
Trade journal	65	66	68	48	30		
Meeting abstracts	1,178	1,022	855	612	615		

ND, data not available.

<sup>&</sup>lt;sup>1</sup> Material Transfer CRADAs.

<sup>&</sup>lt;sup>2</sup> Material Transfer Research Agreements. Involves collaborative research on a specific material.

<sup>&</sup>lt;sup>3</sup> Includes Trust Fund Cooperative Agreements, Reimbursable Agreements, Interagency Agreements, and Non-Funded Cooperative Agreements.

<sup>&</sup>lt;sup>4</sup> Number of published manuscripts.

TABLE 2. Invention disclosures and patenting						
	FY 2016	FY 2017	FY 2018	FY 2019	FY 2020	
Total new invention disclosures <sup>1</sup>	174	169	306	228	188	
University co-owned	58	27	29	39	24	
Non-university co-owned	29	21	16	18	13	
USDA solely owned	87	121	261	171	47	
Scientific discipline:						
Biological materials	19	8	141	86	46	
Life science	73	72	55	47	35	
Chemical	48	43	32	42	31	
Mechanical & measurement	21	28	20	13	16	
Plant patents <sup>2</sup>	5	5	6	2	4	
Plant variety protection <sup>2</sup>	8	13	11	4	4	
Plant breeder's rights <sup>2</sup>	0	0	0	1	0	
Plant public release	28	44	41	33	52	
Based upon CRADA research	25	22	23	14	11	
Total U.S. patent applications filed <sup>3</sup>	92	109	108	85	76	
University co-owned	10	21	31	17	20	
Non-university co-owned	9	23	25	17	20	
USDA solely owned	73	65	52	51	31	
Scientific discipline:						
Life science	50	45	53	29	22	
Chemical	24	38	30	34	40	
Mechanical & measurement	10	7	14	12	8	
Plant patents	6	11	3	4	1	
Plant variety protection	2	8	8	6	5	
Total U.S. patents issued <sup>3</sup>	53	68	61	65	50	
University co-owned	15	22	19	16	12	
Non-university co-owned	12	19	11	21	9	
USDA solely owned	26	27	31	28	22	
Scientific discipline:						
Life science	23	37	29	33	17	
Chemical	13	13	15	17	17	
Mechanical & measurement	9	6	12	4	6	
Plant patents	5	6	2	8	3	
Plant variety protection	3	6	3	3	7	
Foreign patenting						
Total foreign patent applications filed	ND	ND	ND	ND	60	
Total PCT Applications Filed	ND	ND	ND	ND	16	
Total foreign patents issued	ND	ND	ND	ND	3	
ND, data not available. <sup>1</sup> Inventions arising at a Federal laboratory. For FY 2014, also includes the plants protected through Plant Variety Protection.						

<sup>&</sup>lt;sup>3</sup> Includes U.S. patent applications, divisional applications, continuation-in-part applications, provisional applications, and Plant Variety Protection.

TABLE 3. Profile of active licenses							
	FY 2016	FY 2017	FY 2018	FY 2019	FY 2020		
Total active licenses <sup>1</sup>	419	426	460	497	565		
Executed to small businesses <sup>2</sup>	150	155	161	180	236		
Executed to universities	187	186	199	201	208		
Amended in FY	4	4	8	4	4		
Invention licenses <sup>4</sup>	348	351	374	377	393		
Executed to small businesses	112	114	113	114	121		
Executed to start-up businesses	6	6	6	6	6		
Executed to universities	178	176	189	188	191		
Other IP Licenses <sup>5</sup>	71	75	86	120	172		
Executed to small business	38	41	48	66	115		
Executed to start-up businesses	0	0	0	0	0		
Executed to universities	9	10	10	13	17		
Total newly executed licenses	29	38	40	51	93		
Executed to small businesses	9	19	12	25	74		
Executed to start-up businesses	0	0	0	0	0		
Executed to universities	14	9	12	10	7		
Invention licenses	23	29	27	17	30		
Executed to small businesses	6	13	3	7	15		
Executed to start-up businesses	0	0	0	0	0		
Executed to universities	14	8	12	7	3		
Other IP Licenses	6	9	13	34	63		
Executed to small businesses	3	6	9	18	59		
Executed to start-up businesses	0	0	0	0	0		
Executed to universities	0	1	0	3	4		
<b>Total income-bearing licenses</b>	418	425	459	496	564		
Exclusive	289	293	312	312	316		
Partially exclusive	9	6	7	7	7		
Non-exclusive	120	126	140	177	241		
Invention licenses <sup>6</sup>	347	350	373	376	392		
Exclusive	280	283	302	299	300		
Partially exclusive	9	6	7	7	7		
Non-exclusive	58	61	64	70	85		
Other IP licenses <sup>7</sup>	71	75	86	120	172		

<sup>&</sup>lt;sup>2</sup> In the United State, plants may be protected in one of two ways based upon their mode of reproduction: patent (vegetatively reproduced) through the USPTO or variety protection (seed reproduced) through USDA Agricultural Marketing Service. International plants are protected through plant breeder's rights.

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Exclusive	9	10	10	13	16
Partially exclusive	0	0	0	0	0
Non-exclusive	62	65	76	107	156
<b>Total royalty-bearing licenses</b>	145	129	134	125	109
Invention licenses	123	107	112	105	96
Other IP licenses	22	22	22	20	13
Elapsed Amount of Time for Granting Invention Licenses					
Average (months)	4.9	6.1	6.3	5.9	3.6
Median (months)	3.7	5.1	5.5	5.0	2.6
Minimum (months)	0.9	1.3	0.9	1.8	0.8
Maximum (months)	16.0	13.7	24.1	34.9	15.8
Licenses terminated for cause	0	0	0	0	0

ND, data not available.

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<sup>&</sup>lt;sup>1</sup> The rest of the licenses were to medium or large size businesses.

<sup>&</sup>lt;sup>2</sup> A small business, together with its affiliates, must not have more than 500 employees.

<sup>&</sup>lt;sup>3</sup> For the purpose of this report, a startup company is a privately held, U.S. for-profit company operating for less than 5 years and actively seeking financing to commercialize a Federal scientific work product.

<sup>&</sup>lt;sup>4</sup> Invention licenses refer to patents and Plant Variety Protection certifications.

<sup>&</sup>lt;sup>5</sup> Other IP licenses refer to biological materials

<sup>&</sup>lt;sup>6</sup> Invention licenses refer to patents and plant variety protection certificates.

<sup>7</sup> Other IP licenses refer to biological materials

licenses.

TABLE 4. Income from licensing								
	FY 2016	FY 2017	FY 2018	FY 2019	FY 2020			
Total income all active licenses	\$4,784,466	\$5,713,803	\$3,799,1705	\$3,553,446	\$3,243,196			
Invention licenses <sup>2</sup>	\$4,456,054	\$5,377,909	\$3,490,236	\$3,272,205	\$2,968,437			
Other IP licenses <sup>3</sup>	\$328,412	\$325,566	\$308,934	\$281,241	\$274,759			
Total Earned royalty income (ERI)	\$3,633,239	\$3,503,866	\$2,715,861	\$3,171,355	\$2,678,083			
Median ERI	\$3,966	\$3,698	\$3,056	\$3,154	\$4,221			
Minimum ERI	\$5	\$15	\$21	\$ 0.75	\$13			
Maximum ERI	\$818,537	\$769,167	\$265,844	\$573,545	\$279,915			
ERI from top 1% of licenses <sup>4</sup>	NP	NP	NP	NP	NP			
ERI from top 5% of licenses	\$1,811,637	\$1,639,557	\$1,218,975	\$1,579,185	\$1,111,917			
ERI from top 20% of licenses	\$3,043,395	\$2,933,342	\$2,227,058	\$2,655,368	\$2,210,427			
ERI distributed								
Percentage Distributed to Inventors	25	25	25	25	25			
Percentage Distributed to Lab/Agency	0	0	0	0	0			
Innovation Fund <sup>5</sup>	ND	\$483,814	\$618,000	\$833,500	\$898,144			

ND, data not available; NP, data not presented; ERI, earned royalty income.

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ND, data not available; NP, data not presented; ERI, earned royalty income.
 Two of the top revenue-generating licenses expired in FY 2017.
 Invention licenses refer to patents and Plant Variety Protection certifications.
 Other IP licenses. Refer to biological materials licenses.
 Not presented, represents one license.
 Funds are from previous year's revenue.

## 3.6. Downstream Outcomes



## **NUTRITION, FOOD SAFETY, AND QUALITY**

### **National Programs:**

- Human Nutrition, NP 107
- Food Safety, NP 108
- Quality and Utilization of Agricultural Products, NP 306

Dietary carbohydrate intake contributes to reduced stress. Mental stress is linked to risk of chronic diseases. In an 8-week randomized controlled trial that compared effects of a healthy Dietary Guidelines for Americans (DGA)-based diet against the less healthy typical American diet, ARS scientists in Davis, CA, found that the DGA diet, with a higher amount of dietary carbohydrate, resulted in reduced concentrations of a key stress response hormone, cortisol, and dampened stress-induced cortisol reactions. These novel findings provide new evidence suggesting that in the context of a healthy diet, carbohydrate consumption may provide some protection from stress-related disease risk. Furthermore, this apparent stress and cortisol dampening effect could reduce stress-related eating by some individuals and improve their ability to sustain a healthier diet based on the DGA. (NP107, C3, PS3A, Project No. 2023-51530-022-00D)

Obesity dampens immune responses in young women to levels similar to those of elderly. Both obesity and aging are associated with muted immune and inflammatory responses. Limited knowledge, however, exists on differences in the immune system between young and older adults with obesity. ARS-supported scientists in Boston, MA, conducted a study to compare circulating indicators of immunity in young and older women with obesity. Twenty-three young (age 23–43) and 21 older (age 60–83) women with obesity participated. Older women with obesity had significantly fewer circulating immune cells of four specific types than young women. With few exceptions, there was no significant difference in inflammation markers or stimulated lymphocyte proliferation and cytokine production by peripheral blood mononuclear cells between young and older participants. These findings contrast with those previously reported in young and older subjects with healthy weight and call for further investigation into the impact of obesity on premature aging of the immune system. (NP107, C3, PS3B, Project No. 8050-51000-100-00D)

Healthy gut microbes increase muscle strength in the elderly. ARS-funded researchers in Boston, MA, compared the gut microbiome composition in 18 older adults in good physical condition with that of 11 older adults in poor physical condition and observed differences in both sets of individuals. Researchers transferred fecal samples from humans into the guts of microbiome-free mice and found the mice that received transplants from the people in good physical condition had significantly greater muscle strength than mice that received fecal transplants from the people who were in poor physical condition. These results suggest that a role exists between the gut microbiome and maintenance of muscle strength with advancing age. Such understanding may lead to advances in mobility and independence for older adults. (NP107, C3, PS3A, Project No. 8050-51000-104-00D)

Intestinal permeability is affected by gender and genetics in children. A certain amount of absorption by the intestine is essential for life but excess permeability is associated with adverse health consequences. ARS-supported researchers in Houston, TX, studied large numbers of children with normal gastrointestinal function, irritable bowel syndrome (IBS), or functional abdominal pain (FAP), along with their siblings and parents. Several partially absorbable sugar derivatives were administered orally, and amounts recovered in urine over a day measured intestinal permeability. As expected, children with IBS had increased intestinal permeability, but this was not found in those with FAP. Boys had a significantly weaker intestinal barrier than girls and both the siblings and parents of children with higher permeability showed the same pattern of results. Because recent studies show that bacteria in the intestine contribute to liver disease and perhaps obesity, the stronger the gut barrier, the less likely those microorganisms and their potentially harmful products can enter the bloodstream. These data may help explain why boys are more susceptible than girls to liver disease. (NP107, C4, PS4A, Project No. 3092-51000-062-00D)

Diet is a risk factor for type 2 diabetes (T2D) and T2D may impact bone health. Dietary guidance seldom distinguishes between subgroups of individuals, but increasing evidence is showing that a one-size-fits-all approach to diet recommendations is not valid. ARS scientists in Boston, MA, in conjunction with scientists at the Harvard Medical School and the Beth Israel Deaconess Medical Center assessed bone health in Black and White individuals with no diabetes, prediabetes, and early stage T2D. Association between bone health and blood sugar control differed by race. There was no change across the diabetic categories among White individuals; however, bone health was significantly lower in Black subjects with T2D than in those with prediabetes or no diabetes. These findings suggest there may be different associations of blood sugar and bone health in Black and White subpopulations and that

health/diet guidance for the population in general may not be adequate for some subpopulations. (NP107, C3, PS3A, Project No. 8050-51000-099-00D)

Non-inherited changes in DNA due to diet are associated with cardiovascular disease risk factors and all-cause mortality. Although genetics is known to play a role in heart disease and expected lifespan, there are also changes to genetic material that are not inherited. Such changes include binding of methyl groups to DNA, which changes the expression, or activity, of various genes. ARS-supported scientists in both Boston, MA, and Houston, TX, took part in a consortium that analyzed blood samples from 6,662 individuals with European ancestry, 2,702 with African ancestry, and 360 with Hispanic ancestry. In individuals with European ancestry, habitual diet quality was associated with differential methylation levels in sites on DNA of white blood cells, most of which were also associated with multiple health outcomes. These findings demonstrate that integrative genomic analysis of dietary information may reveal molecular targets for disease prevention and treatment that are amenable to improved dietary choices. (NP107, C5, PS5B, Projects Nos. 8050-51000-103-00D and 3092-51000-063-00D)

Neonatal diet alters gut bacteria and metabolite signals in infants. Early nutrition can significantly affect intestinal colonization by normal bacteria and modulate host health through a series of bacterial metabolites that interact with cells of the body. ARS-supported scientists in Little Rock, AR, analyzed fecal samples from infants in an effort to describe the bacteria and their metabolites over the course of the first year of life in babies who were exclusively fed breastmilk or formula. Breastfeeding resulted in increased abundance of bacteria that produce short-chain fatty acids and metabolites that serve as signals in development of the gut and other organs. In addition, bacterial metabolites such as kynurenic acid, which helps optimize immune responses, including inhibiting allergy, were higher in breastfed infants.

These results provide new information about how breastfeeding promotes intestinal and immune health in infants and adds to the scientific basis for the recommendation by the Centers for Disease Control and Prevention that infants should be breastfed. (NP107, C5, PS5A, Project No. 6026-51000-012-00D)

Maternal diet and body fat alter placental DNA methylation. Epigenetic changes, or changes in gene expression rather than alterations of the genetic code itself, provide a possible explanation of how the *in utero* environment programs health throughout the life course. Epigenetic marks can include addition or removal of natural chemicals such as methyl groups on the DNA or the proteins (histones) around which DNA is wrapped. Researchers in Little Rock, AR, compared DNA methylation in placentas from 150 women who were either normal weight or overweight/obese. Both maternal weight status and dietary saturated fat intake were associated with epigenetic changes in placental DNA and many of the modified genes related to fat synthesis, insulin signaling pathways, and DNA packaging. These data indicate that improved diet and weight status can modify placental function and development of the fetus. (NP107, C5, PS5B, Project No. 6026-51000-012-00D)

Data linked across multiple domains are critical for solving problems related to diet and human health. Building bridges between unconnected human nutrition databases is a critical first step to solving these problems, but manually mapping items between databases is expensive and time consuming. The food composition database that underlies the Automated Self-Administered 24-Hour (ASA24) dietary assessment tool includes 65 nutrients; however, linking this database to other similar databases could provide estimates for nearly 100 additional nutrients. ARS scientists in Davis, CA, and collaborators at the University of California, Davis, used machine learning and database mapping methods to estimate the dietary intake of lactose, which existed only in the unconnected database. Food items in the two databases were acceptably matched based on their nutrient profiles and text

descriptions. Compared to manual mapping of food items by experts, this method reduced the time required for estimating lactose by 99.7 percent. These results suggest that unconnected food composition databases may be linked using computer-assisted methods, thereby expanding the ability of researchers to assess many additional nutrients not available in single databases. This increased capacity benefits the nutrition research community by expanding the tools they have available for their studies, and will also benefit nutrition professionals, and eventually consumers, by improving dietary guidance for Americans. (NP107, C2, PS2A, Project No. 2032-51530-026-00D)

National survey data and related databases for recent years are available. ARS has partnered with the Centers for Disease Control and Prevention on producing dietary data from the National Health and Nutrition Examination Survey (NHANES) for many years. ARS scientists in Beltsville, MD, released nationally representative survey data for 2017–2018 as part of What We Eat in America, NHANES. In addition, the 2017–2018 update of the Food and Nutrient Database for Dietary Studies was released. Tables providing national dietary intake estimates compared to nutrient requirements were also made public. Additionally, seven dietary data briefs were published providing information for nutrition policy and education on topics such as intake of added sugars; vegetable, fruit, and dairy intake; late evening snacking; and convenience stores as sources of foods and nutrients. Regular updates of nationally representative data are essential for the Federal Government to produce dietary guidance, including the Dietary Guidelines for Americans, which is published jointly by USDA and HHS. (NP107, C2, PS2B, Project No. 8040-53000-020-00D)

Daily zinc supplementation affects immune function in Laotian children at risk of deficiency. Zinc deficiency, common among children in Southeast Asia, impairs immune function and increases the risk of infectious disease. ARS scientists in Davis, CA, worked with collaborators from the University of

California, Davis, Khon Kaen University in Thailand, and Lao Tropical and Public Health Institute in Lao People's Democratic Republic, to determine how the method of supplementation affected immune function in rural Laotian children. Children (age 6–23 months) received one of four zinc treatments (placebo, daily zinc, daily zinc with other micronutrients, or only when children were treated for diarrhea) for 9 months. The results of the study showed that daily zinc tablets resulted in significant improvement in immune endpoints relative to the placebo group. Immune cell concentrations in blood decreased in children who received zinc supplements, suggesting that zinc may have helped control an underlying infection. These results provide important information for public health experts in Laos and internationally regarding how best to supplement zinc to prevent deficiency. (NP107, C3 PS3A, Project No. 2032-51530-026-00D)

## First ever expert advice to parents and caregivers on healthy eating behaviors in young children.

Researchers know that to prevent childhood obesity, how children eat may be as important as what they eat. In addition, we know that the first 5 years of life are a critical period for helping children learn how to regulate their food intake to match their energy needs. ARS-supported researchers in Houston, TX, led a partnership with other scientific experts and the American Heart Association to release their first ever scientific statement giving advice on how to foster healthy eating behaviors in children under 5 years of age. This guidance will broadly reach parents and caregivers across the country and provide strategies for healthy eating behaviors with the goal of reducing childhood obesity. (NP107, C4, PS4A, Project No. 3092-51000-063-00D)

**Fruit pigment metabolites cross the blood-brain barrier.** The blood-brain barrier is a special feature of the circulatory system that protects the brain from many factors circulating in the blood. There has been much speculation about whether potentially health-promoting dietary compounds can cross the

barrier to reach the brain and improve mental performance. ARS scientists in Beltsville, MD, collaborated with colleagues in several European universities to show for the first time that flavanols, which are abundant in fruits, cocoa, and pulses, are metabolized by the intestinal bacteria to a form that is absorbed and circulates in the blood that can be recovered from brain tissue. The results of a series of studies using rats and pigs clearly demonstrate the permeability of the blood-brain barrier and provide additional evidence that a gut-brain axis exists and that there are potential health benefits from eating a variety of plant foods. (NP107, C3, PS3A, Project No. 8040-51530-058-00D)

Eating enough protein at every meal decreases odds of functional disability. Functional ability is associated with quality of life, health, and mortality. Given our rapidly growing aging population, preserving functional ability is a public health priority. Strategic intake of dietary protein has demonstrated promise for preventing functional disability during aging. ARS scientists in Grand Forks, ND, in collaboration with scientists at North Dakota State University, found an association in a large epidemiologic study that eating at least 1 gram of protein for every kilogram of body weight daily and distributing the protein evenly across all eating occasions decreased the odds for functional disability by up to 61 percent, and the greater the number of eating occasions containing enough protein, the stronger the benefit. The study involved 8,000 people who were at least 60 years old. Results suggest that consuming protein and spreading it across all meals is associated with better physical functioning in older adult patients. (NP107, C3, PS3B, Project No. 3062-51000-057-00D)

High intake of plant pigments is associated with reduced risk of Alzheimer's disease. Alzheimer's disease is the cause of 60 to 80 percent of dementia cases, making it a major public health challenge for which there is no effective therapy. ARS-supported researchers in Boston, MA, followed 2,809 men and women older than age 50 for an average of 20 years as part of the Framingham Heart Study. Specific

fruits and vegetables rich in plant compounds known as flavonoids and associated with significantly reduced risk of Alzheimer's dementia included blueberries, strawberries, and red wine. Apples, pears, oranges, bananas, and tea also showed some beneficial associations. These results suggest that adding certain fruits to the diet may be linked to a reduced risk for the most widespread type of senility and support the recommendation of the Dietary Guidelines for Americans to eat a variety of fruits and vegetables. (NP107, C3 and 5, PS3B and 5A, Project No. 8050-51530-014-00D)

People with certain genes are more likely to gain weight when consuming sugar-sweetened beverages. Consuming sugary drinks is associated with obesity and obesity-related diseases, but the biological mechanism that connects sugar-sweetened beverage intake to obesity is not completely understood. ARS researchers in Boston, MA, examined the relationship of biochemical compounds found in the blood of participants in the Boston Puerto Rican Health Study as it related to their intake of sugar-sweetened beverages and body mass index. The scientists identified 28 compounds, many of them implicated in fatty liver, that linked sugar-sweetened beverage intake with obesity. Those compounds were mainly in two genetic pathways of lipid metabolism. These findings suggest that drinking sugar-sweetened beverages disrupts the metabolism and leads to an increased risk of obesity in persons with specific versions of genes. Reducing consumption of sugar-sweetened beverages would contribute to reducing the risks of obesity and fatty liver disease that currently affect millions of Americans. (NP107, C4, PS4A, Project No. 8050-51000-107-00D)

A mega-method to analyze contaminants in foods. Analysis of foods for the presence of pesticides, veterinary drugs, and environmental contaminants is necessary for public health. In 2003, ARS developed the QuEChERS approach to sample analyze pesticide residues in foods. This method has now become the primary and gold standard used worldwide in chemical residue analysis. Instrumentation and

technology have continued to improve in the past 17 years, creating a need to update the QuEChERS method. Consequently, ARS scientists in Wyndmoor, PA, have now developed and validated the "quick, easy, cheap, effective, rugged, safe, efficient, and robust" (QuEChERSER) mega-method using mass spectrometry to analyze pesticides, veterinary drugs, and environmental contaminants in foods. So far, the new QuEChERSER mega-method has been validated for up to 349 diverse analytes in fish, bovine, caprine, and ovine muscle; hemp products; and fruits and vegetables. Once implemented internationally, QuEChERSER is expected to eventually supplant QuEChERS as the primary method for monitoring a wide array of chemical contaminants in foods. (NP108, C1, PS4, Project No. 8072-42000-080-00D)

Colistin-resistance in U.S. animal-origin food. Colistin, also known as polymyxin E, is a last-resort antibiotic against drug-resistant Gram-negative bacterial infections. Recently, a mobile colistin resistance gene, mcr-1, was discovered in clinical and animal samples. The prevalence of mcr-1-mediated colistin resistance has never been investigated in U.S. animal products. To fill this gap, ARS researchers in Albany, CA, screened more than 5,000 domestic food samples (chicken rinse, ground beef, beef trim, poultry, raw pork, and catfish) randomly collected by the USDA Food Safety Inspection Service for the presence of mcr-1 using a novel method developed by ARS that combines an enzymelinked immunosorbent assay with real-time polymerase chain reaction methods. The screening data revealed a very low prevalence (0.02 percent) of the mcr-1 gene in tested samples. Subsequent whole genome sequence analysis on the single positive isolate revealed that the mcr-1 gene resided on an IncI2 plasmid. This study was the first systemic and large-scale investigation of mobile colistin resistance in U.S. food animal products, and the information will be important for trade-related food safety risk assessments. (NP108, C1, PS3, Project No. 2030-42000-049-00D)

A novel strategy for estimating Salmonella contamination levels in raw ground beef. Salmonella is a leading cause of foodborne illness worldwide. In spite of the use of numerous process controls in food production industries, there has been little progress in decreasing the occurrence of Salmonella food poisoning over the past decade. This is in part because current testing methods indicate only the presence or absence of Salmonella, but they do not measure how much contamination is in a tested product. To address this need, ARS scientists in Clay Center, NE, developed a novel strategy for rapidly estimating Salmonella contamination levels in raw ground beef based in the same amount of time that it takes to detect Salmonella in enrichments using two different commercial molecular detection methods. The ability to detect high levels of Salmonella contamination will enable meat companies to improve their process controls and remove more highly contaminated products from the food chain. This will improve the safety of beef and decrease the incidence of human exposure to levels of Salmonella that cause disease. (NP108, C1, PS3, Project No. 3040-42000-017-00D0)

Predictive models to identify antifungal compounds. Antifungal chemicals are often used to reduce crop spoilage and reduce the occurrence of mycotoxins. However, safer, better antifungal agents are needed. Some potential antifungal agents are phenolic compounds that have many uses due to their consumer-friendly properties. To aid in the selection of better antifungal compounds, ARS scientists in Peoria, IL, applied computational artificial intelligence and machine learning methods to develop mathematical models that identified chemical properties of phenolic compounds that reduce contamination by mycotoxin-producing fungi. Two of the antifungal compounds evaluated, thymol and carvacrol, are components of essential oils of many plants, including the popular culinary herb thyme. These models will help toxicologists, microbiologists, and chemists discover better antifungal agents to benefit the food industry. (NP108, C1, PS6, Project No. 5010-42000-049-00D)

Survival of bacterial pathogens in manure-amended soils. Fresh produce, especially leafy greens, has been implicated as a source of several major outbreaks of foodborne illness in the United States.

Manure-amended soils continue to represent a potential source of bacterial pathogen contamination. The Food and Drug Administration (FDA) Produce Safety Rule, as part of the Food Safety Modernization Act, prohibits the use of untreated manure within 90 or 120 days prior to the harvest of edible produce crops. To examine the role of manure types in produce contamination, ARS scientists in Beltsville, MD, in collaboration with the FDA and academia, collected manure/produce data from 12 field trials conducted over 4 years at 3 separate locations. The data were used to identify factors affecting the survival of *Escherichia coli* in manure-amended soils. The studies showed that poultry litter supported longer survival of *E. coli* than dairy or horse manure. Days of rainfall and soil moisture content affected *E. coli* survival in manure-amended soils. These results assisted the FDA in developing models to determine the appropriate interval between application of raw manure and harvest of edible crops to minimize fresh produce contamination, thus making produce safer for consumers (NP108, C1, PS6, Project No. 8042-32420-006-00D)

Safe use of chlorine dioxide to sanitize produce and eggs. Chlorine dioxide (ClO2) gas is very effective at eliminating microbiological contaminants from a variety of fruits, vegetables, melons, seeds, and even eggs. Although the gas has been proposed for sanitizing human foods to eliminate pathogens and rot organisms, it has not been approved by the Food and Drug Administration or Environmental Protection Agency for use on foods other than tomatoes and cantaloupe. This non-approval is because chemical residues of ClO2 have not been described. ARS researchers in Fargo, ND, demonstrated that nearly all the residue deposited on the surfaces of eggs, avocados, onion, and sweet potato after the use of ClO2 for sanitation is a harmless chloride ion. The studies also found that chlorate, a byproduct of ClO2, was present in low quantities and could serve as a useful marker of ClO2-treated products. The

results show that chemical residues are not a major obstacle for the commercial development of cost-effective ClO2 gas technologies to safely eliminate pathogens and rot organisms from a variety of produce and eggs. (NP108, C1, PS4, Project No. 3060-32420-001-00D)

Combining cold plasma and hydrogen peroxide as a postharvest intervention. Fresh fruits and vegetables are a major source of essential nutrients for humans. However, these products are subject to contamination by both pathogens and spoilage microorganisms, which reduces their safety, quality, and nutritional value. Fresh produce is often consumed raw or after minimal processing, and pathogen contamination can present higher risks of outbreaks of foodborne illness. Because there are food safety uncertainties along the supply chain, postharvest treatments are essential in reducing the risk of pathogen contamination and minimizing the risk of microbial spoilage. ARS scientists in Wyndmoor, PA, combined cold plasma and hydrogen peroxide aerosols to produce highly reactive radicals that reduced the populations of bacteria on fresh fruits. Applying cold plasma to hydrogen peroxide increased the effectiveness of hydrogen peroxide aerosols and killed almost 100 percent of Salmonella and Listeria on surfaces of apples, cantaloupe, and tomatoes. This new process did not affect appearance, color, texture, or nutritional quality of the produce. The outcome of this work has a direct application to the produce industry, and ARS is collaborating with industry partners to evaluate the commercial implementation of the new intervention technology. (NP108, C1, PS3, Project No. 8072-41000-101-00D)

A novel aqueous ozone intervention against *Escherichia coli* O157:H7 on fresh beef. Ozone is a naturally occurring water-soluble gas that is an effective germicide and has been approved as a sanitizer for food-contact surfaces and food products. *E. coli* O157:H7 is a foodborne bacterial pathogen that has been implicated in many cases of meat-associated human illness in the United States. The last step in

beef processing is to rapidly cool the carcass to 35°F, and this is accelerated by applying periodic sprays of cold water. ARS scientists in Clay Center, NE, evaluated a new nanobubble technology that creates a stable, high concentration of aqueous ozone for its effect on pathogenic *E. coli* that can be present on beef during spray chilling. The results indicated that the novel ozone spray was 80 percent more effective in reducing pathogenic *E. coli* than water alone. Because beef carcasses are usually chilled under recurring sprays of water for 6–8 hours, by adding ozone this process can now be a continued antimicrobial step leading to safer end products for consumers. (NP108, C1, PS5, Project No. 3040-42000-018-00D)

A rapid test for masked toxins in wheat. Trichothecenes are a group of fungal toxins (mycotoxins) that can contaminate oat, wheat, barley, and corn, and cause substantial economic losses worldwide. Trichothecenes are toxic to humans and animals, and upon consumption, the toxin inhibits ribosomal protein, DNA, and RNA synthesis; mitochondrial functions; and cell division while simultaneously activating a cellular stress response. As part of efforts to improve monitoring of these trichothecene toxins, ARS scientists in Peoria, IL, in collaboration with the Institute of Sciences of Food Production in Bari, Italy, developed a new method to detect trichothecenes in wheat. Trichothecenes are also toxic to plants, but plants can protect themselves from the toxins by attaching a sugar residue to a trichothecene molecule, which makes them less toxic. The plant toxin derivatives are called masked mycotoxins, and are difficult to detect. During the human digestion process, the original toxin may be released from the masked state, resulting in mycotoxin poisoning. The new toxin/masked mycotoxin detection method is rapid, sensitive, and convenient and will be used to monitor trichothecenes and their modified forms in wheat. Improved monitoring for the trichothecenes and their masked forms can be used to reduce exposure to these toxins by diverting the contaminated food product from the food supply. (NP108, C1, PS6, Project No. 5010-42000-049-00D)

Wireless, high-resolution, time-temperature measurement of foods. Foods, especially ready-to-eat foods available for consumers via retail outlets, may undergo temperature fluctuations due to faulty refrigeration. These fluctuations may induce growth of pathogens and spoilage microorganisms.

Continuous temperature monitoring is required to avoid any food safety or quality concerns. ARS-funded scientists at the Center for Food Safety Engineering at Purdue University in West Lafayette, IN, developed a system that can be integrated in delicatessen cases and is capable of acquiring temperature measurements using low-cost tags that can be attached to food packages. The system provides high-resolution temperature measurement that can be integrated into the "Internet of Things" (IoT) through Bluetooth communication capabilities. Integrating the system in retail deli cases can enable real-time risk assessment of stored products with direct notification of the management when irregular storage conditions occur. Implementation of such a system will enhance the safety and quality of ready-to-eat foods. (NP108, C1, PS3, Project No. 8072-42000-077-00D)

Flowing steam decontamination of broiler transport cages. Live-haul cages are used to transport broilers from the farm to a processing facility. These cages are large and expensive; therefore, companies have a limited supply and continually reuse the same cages. *Campylobacter*, a significant human foodborne pathogen, can be readily detected in the feces of broilers from a Campylobacter-positive flock. Feces left in a cage by broilers that carried *Campylobacter* can contaminate the next broilers placed in the same cage. Studies have previously shown that water spray and sanitizing broiler transport cages is logistically complicated, physically difficult, water intense, and largely ineffective to eliminate *Campylobacter*. ARS researchers in Athens, GA, tested steam as a means to decontaminate transport cage flooring, which resulted in an approximately 99 percent reduction in the number of *Campylobacter* bacteria detected. When the steam treatment was preceded by a 15-second water spray, the *Campylobacter* reduction was improved to 99.99 percent compared with untreated cages. Although

Campylobacter was not completely eliminated, steam shows potential as an effective method for sanitizing broiler transport cages and to control transfer to previously negative broilers. Lowering the number of Campylobacter bacteria on live broilers entering the processing plant would be expected to lessen contamination of fully processed poultry meat products and reduce consumer exposure to Campylobacter. (NP108, C1, PS5, Project No. 6040-32000-009-00D)

Eliminating *Campylobacter* in chicken livers. Campylobacter species are significant human foodborne bacterial pathogens specifically associated worldwide with poultry and poultry products. Foodborne outbreaks of campylobacteriosis, the disease ascribed to the pathogen, can often be traced to pâté or mousse prepared from undercooked chicken liver. *Campylobacter* is readily detected on fresh raw chicken livers in the processing plant and at retail. Infections have become so prevalent in the last several years that the USDA Food Safety and Inspection Service was prompted to call for interventions to decontaminate chicken liver. ARS researchers in Athens, GA, tested heat and cold treatments to lessen *Campylobacter* contamination of fresh chicken livers. Immersion in 60°C water for 5 minutes resulted in significantly lowering *Campylobacter* numbers. Forty-eight hours at –25°C in a household freezer was moderately effective. When heat and freezing were combined in series, a nearly 99 percent decrease in the number of naturally occurring *Campylobacter* on both the surface and within inner tissue of chicken liver was achieved. Thus, a mild heat process followed by freezing can be recommended for presentation of poultry livers at retail or home and will reduce consumer exposure to *Campylobacter*. (NP108, C1, PS5, Project No. 6040-32000-009-00D)

**Simple and portable test for amatoxins.** Amatoxins are lethal toxins found in certain mushrooms, particularly the death cap mushroom, which cannot be eaten. Because many inedible mushrooms are physiologically similar to edible mushrooms, many people (and animals) are sickened (and many die)

from mistaken consumption. Additionally, most medical and veterinary personnel have difficulty determining which poison a human or animal might have ingested. ARS researchers in Albany, CA, developed an antibody-based immunoassay that can detect as little as 10 ng/ml of amatoxins in both mushroom and urine samples without the need for specialized equipment. The detection of amatoxins in urine samples correlated very well with the traditional methods of using liquid chromatography-mass spectrometry. The speed of analysis and lack of needing trained personnel and expensive instrumentation will offer a quick way to directly diagnose amatoxin-specific mushroom poisonings. The new immunoassay is currently being used and tested by several animal clinics through material transfer agreements and informal collaborations. A patent is pending for the new immunoassay. (NP108, C1, PS3, Project No. 2030-42000-049-00D)

Escherichia coli (STEC) are a group of foodborne bacterial pathogens that can be transmitted to humans mainly through food and water. STEC strains are implicated in more than 270,000 cases of human illness annually in the United States. STECs naturally reside in cattle, and are found in natural creek sediments that are affected by runoff and fecal pollution from agricultural and livestock practices. ARS researchers in Albany, CA, detected STECs from the water-sediment interface of two creeks in the Salinas River Valley of California, an area that is known to be associated with STEC-derived foodborne illness. Shiga toxin-encoding genes were not directly detected in the metagenomes of samples that were culture-positive for STEC, indicating that STEC was present at very low levels in those sediments. Furthermore, there were no significant differences in the abundance of human or cow-specific microbiome sequences between the control and sampling sites, implying a natural dilution of the human inputs. This study provides metagenomic parameters for the Food and Drug Administration to use in

enforcing the Produce Rule within the Food Safety Modernization Act. (NP108, C1, P12, Project No. 2030-42000-050-00D)

Vaccination of cattle and impact on intestinal microbiota. Vaccines targeting the bacterial pathogen *Escherichia coli* O157:H7 (O157) in cattle have the potential to reduce O157 colonization and thus reduce carcass contamination. However, non-O157 *E. coli* are part of the normal microbiota, and vaccination against O157 *E. coli* may impact numbers and types of normal bacteria (microbiota) found in cattle intestines. Because the intestinal bacteria play a critical role in animal health, alterations induced by vaccination could affect an animal's immune response and overall health. ARS researchers in Ames, IA, evaluated the impact of O157 vaccination and O157 colonization on the diversity of intestinal microbiota to gauge potential unforeseen consequences of O157 vaccination. Microbiota analysis of fecal samples (which contains intestinal bacteria) from vaccinated and nonvaccinated cattle indicated a significant correlation between vaccination and alterations in intestinal bacterial populations. Whereas vaccination may be a strategy to limit O157 in cattle, unforeseen consequences of changes in beneficial bacterial populations warrant further consideration. (NP108, C1, PS1, PS2, PS3, and PS5, Project No. 5030-32000-112-00D)

Simple, low-cost CCD camera system for active abrin detection. Abrin is a natural but lethal toxin produced and found in the seeds of the rosary pea (*Abrius precatorius* L.) The toxin has a similar mode of action to that of the select agent ricin, which is found in the seeds of the castor bean plant. There are many ways to test for the presence of abrin, but few assays can distinguish between the active (lethal) and inactive (nonlethal) form of the toxin. ARS researchers in Albany, CA, developed and built a simple, low-cost charge-coupled device (CCD) camera and applied it to cell-based assays. In presence of active abrin, the cells either produced a color change or a change in their fluorescent glow. The new

fluorimetric method was able to detect as little as 0.1 pg/mL of active abrin. This simple and inexpensive method directly adds to the arsenal of tools for the accurate detection of abrin poisoning, which may occur, for example, in cases of intentional food adulteration/contamination. (NP108, C1, PS3, Project No. 2030-42000-049-00D)

Bioinformatic tool for bacterial genome analysis. The development and implementation of nextgeneration sequencing has significantly improved our understanding of the bacterial genome architecture. However, there are still multiple bioinformatic hurdles to maneuver before a bacterial species becomes an informative annotated genome. Currently, bacterial bioinformatics generally entails using several stand-alone tools that make the process cumbersome and prone to specialist/human error. Developing technologies to eliminate these errors is a critical research need. ARS researchers in Athens, GA, and Colorado State University developed Reads2Resistome, a bioinformatic tool that streamlines this arduous effort. Reads2Resistome allows users with experience using basic Linux commands to analyze bacterial genomes using either short-read or long-read sequencing technologies. Reads2Resistome takes sequence reads as input and performs assembly, annotation, and genome characterization with the goal of producing an accurate and comprehensive description of the bacterial genome. Included in the analysis is determination and collection of all the antibiotic resistance and virulence genes, and other resistance elements within the main chromosome, or other elements such as plasmids or bacteriophage. The pipeline is executable on both Mac and Linux operating systems and is well suited for institutions and organizations that maintain or have access to a high-performance cluster for analyzing big data. Reads2Resistome is the first pipeline to our knowledge that performs both genome assembly and in-depth genome characterization. The new technology has been made publicly available on GitHub and is accessible to USDA researchers via SCINet. (NP108, C1, PS3 and PS7, Project No. 6040-32000-009-00D)

Predicting Salmonella prevalence. Salmonella contamination of poultry and poultry products remains a critical concern for the USDA Food Safety Inspection Service. Farmers and inspectors alike need a way to predict the prevalence of Salmonella during the pastured poultry farm-to-fork continuum so that the correct and appropriate interventions can be applied. ARS researchers in Athens, GA, used random forest modeling, farm-management data obtained through questionnaires, and meteorological data to develop and validate algorithms that were effective at predicting Salmonella prevalence. The predictive modeling showed that years farming, broiler flock age, and dominant feed components were major farm management drivers of Salmonella prevalence in preharvest samples, whereas dominant feed components was the most relevant driver of Salmonella prevalence in postharvest samples. Average temperature, humidity, and high wind gust speeds prior to sampling were the meteorological variables that most closely correlated to Salmonella prevalence in preharvest samples. These data provide stakeholders with target variables to monitor to determine potential Salmonella food safety risks within their management systems. (NP108, C1, PS3, PS5, PS6 and PS7, Project No. 6040-32000-011-00D)

Smartphone-based spectrometer. Industry and regulatory inspectors are required to monitor products for safety as they proceed through the food production and processing continuum. Normally samples are taken and sent to an internal or external laboratory for analysis using various types of assays. A major limitation of many food safety assays is that they require expensive equipment found only in centralized laboratories. Both industry and inspectors would prefer, where possible, that samples obtained in the field be analyzed on site, and data immediately be made available. ARS-funded scientists at the Center for Food Safety Engineering at Purdue University have developed a smartphone-based spectrometer that can resolve the visible range of spectrum in transmission mode and can be used to analyze many types of food safety assays. The overall cost of the spectrometer is only \$200 and functions with an app that can visualize, record, and analyze the visible spectrum. The outcome is that this device could be

incorporated into many types of assays with visual readouts to allow data to be used at the point the sample is taken, simplifying the assay process, and thus reducing the time required to obtain a result and transfer the data. (NP108, C1, PS3 and PS4, Project No. 8072-42000-077-00D)

COVID antiviral cotton facemasks. During the COVID-19 enforced maximal telework period ARS researchers at New Orleans, LA, in collaboration with a medical trauma wound dressing company, revealed that a jointly developed cotton nonwoven product exhibited antiviral activity. The natural compound in cotton (hydrogen peroxide) was discovered by ARS and tested by a contract company and was found to have antiviral activity based on a molecular model of SARS-CoV-2 virus. The product exhibited 99.999 percent antiviral activity after 1 hour of contact with the fabric. Nonwoven cotton will be studied with collaborators to determine its ability to inhibit COVID-19, which is caused by the current SARS-CoV-2 virus. Following testing by a secondary company to obtain a Food and Drug Administration-approved ISO test for antiviral textiles, the company plans to develop a prototype for use in facemasks. (NP306, C2, PS2a, Project No. 6054-41430-007-00D)

Removing plastic contamination and increasing cotton's value. Plastic contamination is the single biggest threat to the U.S. cotton industry to date. According to the USDA Agricultural Marketing Service, most of the plastic contamination in test samples from ginned cotton in the United States originates from plastic material used to wrap the harvested cotton modules formed by state-of-the-art cotton harvesters. Plastic contamination is the major reason for the loss of the premium grade status.

U.S. grown cotton was once well received on the international market for its reputation as the world's reliable source of contaminant-free natural fiber. On an annual basis the loss is more than \$750 million.

ARS researchers in Lubbock, TX (with assistance from ARS researchers in Las Cruces, NM), developed a low-cost system that identifies and removes plastic and other contaminants in harvested cotton before

being ginned. This system, commercially known as VIPR (for Visual Inspection and Plastic Removal), uses imaging sensors from the cell phone industry with low-cost embedded microcontrollers to identify contaminants. When a contaminant is detected, a pneumatic system blows the contaminant out of the cotton and onto the floor. Commercial testing shows that the system can operate with detection/removal efficiency of more than 90 percent. This technology was developed, tested, and successfully transferred to a commercial partner and is now being sold domestically and internationally. This system will return "premium grade" status to U.S. cotton and over the next decade is expected to earn the industry more than \$7 billion. (NP306, C2, PS2a, Project Nos. 3096-21410-008-00D and 3050-41000-009-00D)

Discovery of a standard reference for wheat "falling number" quality determination. Falling number is an 80-year-old method used the world over to measure the quality of harvested wheat. There has long been a need to identify a stable reference material to standardize the precision and accuracy of machines that calculate falling numbers. ARS engineers in Beltsville, MD, determined that corn starch is an excellent material to serve as a substitute for harvested wheat because of its high precision in falling number tests, long shelf life, and low cost. Government regulatory agencies in Canada, the USDA Agricultural Marketing Service, and private grain-handling and milling companies are, for the first time, developing the acceptable limits for a worldwide standard chart, based on this standard reference, to reliably determine wheat falling number. (NP306, C1, PS1a, Project No. 8042-44000-001-00D)

Biobased cat litters made from renewable low-value plant fibers. ARS scientists in Peoria, IL, using low-value, invasive Eastern red cedar (ERC) wood chips, developed new absorbents and pest control products. ERC lumber and sawdust were processed for optimal use as a biobased absorbent that contains essential oils shown to be repellant and/or toxic to fleas and ticks. An absorbent formulation consisting of 10 percent ERC biochar, 84 percent ERC wood fibers, 4 percent guar gum, and 2 percent mineral oil

provided excellent suppression of odors, including the major cat urine odor compound, and had physical and chemical properties equal to or superior to the top three biobased cat litters currently on the market. A U.S.-based pet care company has signed a confidential agreement to work with ARS scientists on this cat litter and an invention disclosure has been approved by the ARS Chemical Patent Committee. (NP306, C1, PS1c, Project Nos. 5010-41000-167-00D and 5010-41000-169-00D)

Unlocking the genetic resistance of soft winter wheat preharvest sprouting (PHS). PHS is the germination of wheat grains in the field before harvest following 2 or more consecutive days of rain with cool temperatures. These weather conditions occur in the soft winter wheat growing region about once every 2 years and farmers can lose approximately 30 percent of their crop value, which translates into approximately \$420 million annually in the United States. Much is known about the biochemical process of PHS but little is understood about the genetics of PHS. Because its genetic nature is not well understood, few markers exist for breeders to use in developing resistant lines. ARS scientists in Wooster, OH, completed a survey of PHS resistance in a population of nearly 200 diverse soft wheat breeding lines over 3 years and over 2 years in a second, and a more diverse population of older varieties. Nine soft winter wheat varieties were identified as PHS-tolerant over multiple years under PHS-inducing conditions. These findings provide important information for breeders about varieties with tolerance to PHS and the potential for future breeding of PHS resistance. (NP306, C1, PS1a, Project No. 5082-43440-001-00D)

First ever control of blueberry fruit rot. Control of postharvest blueberry fruit rot is important to maintain domestic and international marketing and shelf life, and to reduce fruit loss and waste. To date no products have been registered specifically to control blueberry fruit rot. ARS researchers in Parlier, CA, tested reduced-risk fungicides as a preharvest treatment and a postharvest continuous ozone

fumigation to control blueberry fruit rot. A mixture of the fungicides fludioxonil and cyprodinil was found to significantly reduce gray mold and *Alternaria* rot, the two most important postharvest rot diseases of blueberries grown in California. Continuous ozone fumigation at low doses significantly reduced gray mold and its fruit-to-fruit spread. These treatments provide new tools for control of commercial postharvest fruit rot diseases of blueberries. (NP306, C1, PS1a, Project No. 2034-43000-039-00D)

ARS develops the first automated peanut sampling system. Every load of peanuts a farmer delivers for sale is sampled and graded by the USDA Agricultural Marketing Service (AMS) to determine its quality and value. Each load is parked beneath an overhead pneumatic sampler where human operators crawl through the truckload of peanuts to insert a probe up to 15 times in specified locations to obtain samples for testing. ARS engineers in Dawson, GA, designed and installed an instrumentation and control system that automatically senses the load of peanuts under the sampler, locates the trailer, and controls the sampler to probe the trailer according to the prescribed probe patterns. AMS personnel conducted tests for 2 years and have approved the control system for installation at commercial peanut facilities for official sampling. This device reduces the reliance on seasonal labor and improves the consistency and accuracy of the sample used for determining peanut quality and value. (NP306, C1, PS1a, Project No. 6044-41430-006-00D)

High-value gluten-free fiber from grapefruit citrus peel waste. When citrus fruits are processed into juice the waste material is usually converted into low-monetary-value animal feed and molasses in a process that is energy demanding and costly. This process destroys compounds within the residues that, if recovered, can be used to make high-value products such as gluten-free fiber. Low-cost and low-energy-use steam explosion systems have been used in the past to recover valuable compounds from

orange juicing waste residues but never from grapefruit juicing residues. As part of a research agreement with an industry partner, ARS scientists in Fort Pierce, FL, conducted steam explosion on grapefruit processing residues and successfully recovered gluten-free fiber, high-value pectin, phytonutrients, and essential oils. These scientists continue to interact with stakeholders interested in using steam explosion on an industrial level for processing grapefruit peel waste residues at their facilities into high-value bioproducts. (NP306, C1&2, PS1a&2b, Project No. 6034-41000-017-00D)

Making pulses a novel, issue free, food ingredient. Pulses (dried beans) provide valuable health benefits in the diet but are a relatively minor component in the U.S. food economy due to issues such as long cooking time, beany taste, flatulence, and abdominal discomfort. New processing methods are needed to provide pulse-based functional food ingredients in a form that facilitates their incorporation into various food products while minimizing negative characteristics. ARS scientists in Peoria, IL, successfully used jet-cooking to separate navy bean flour into readily obtained water soluble and insoluble fractions while leaving behind almost all the flatulent sugars and the "beany" taste. The researchers discovered the insoluble fractions could serve as a fiber and protein ingredient to fortify bread or spreadable food products, while the soluble fraction has good emulsification and foam properties, making it a potential substitute for egg whites. Because the isolated fractions can be dried and reconstituted or used directly, food producers and consumers can readily adapt them into their manufacturing or food preparation processes. (NP306, C1, PS1b, Project No. 5010-41000-168-00D)

**Potato postharvest quality evaluations and release of new potato cultivars.** Acceptable processing quality after storage is an essential attribute of a successful potato variety. The standardized evaluation procedures developed and used by ARS scientists in East Grand Forks, MN, a worksite of the ARS research unit in Fargo, ND, have been an important component of the overall process for evaluation and

release of new cultivars by Federal and State cooperators nationwide. In the past year, in support of Federal and non-Federal public breeding/screening programs, 139 advanced breeding lines were analyzed for storage/processing quality at multiple storage temperatures and durations. Since 2015, 17 chip clones and 14 fry processing clones identified in East Grand Forks to have superior storage quality have advanced through rigorous national variety testing platforms aimed at providing potato industry stakeholders a high-quality processed potato product throughout storage. Data from these analyses have contributed to the national release of new potato varieties with superior processing quality throughout storage. (NP306, C1, PS1a, Project No. 3060-21430-007-00D)

ARS annual Hard Winter Wheat Crop Quality Survey. ARS scientists in Manhattan, KS, completed the annual Hard Winter Wheat Crop Quality Survey involving the evaluation of more than 600 individual and more than 100 composite, hard winter wheat samples for milling and baking quality. The resulting data were posted in real time to a web page managed by Plains Grain, Inc., as the harvest progressed; the data were also used by U.S. Wheat Associates in its final annual report for domestic and international export customers. (NP306, C1, PS1a, Project No. 3020-44000-026-00D)

Corn starch: It's an emulsifier and a pesticide. An emulsifier allows two normally insoluble materials to become a stable mixture. Many industrial emulsifiers use carcinogenic or highly hazardous ingredients. As a result, there is a constant need for improved industrial emulsifiers that provide biobased and safe alternatives. ARS scientists in Peoria, IL, have developed an economical emulsifier that uses corn starch and a vegetable oil. This new emulsifier (called an AIC) forms suspensions of oil in water, which are stable for months and makes water slicker, allowing the AIC-water solutions to lubricate parts and allowing for efficient cleaning. In addition, the AIC can control Gram-positive bacteria, yeast, mold, fungi, and some insects, including termites. The ability of corn starch to function

as both an emulsifier and pesticide is highly attractive and has given it a higher value. This new technology allows for the replacement of imported emulsifiers or those that use hazardous ingredients or processes. These new products are being promoted by industry resulting in new applications for corn starch and benefiting corn producers, processors, and consumers. (NP306, C1 & C2, PS1c, & PS2b, Project No. 5010-41000-166-00D)

Treatment of poison ivy dermatitis. ARS researchers in Oxford, MS, in collaboration with ElSohly Laboratories, Inc. and university researchers, are developing preventive treatments for poison ivy dermatitis. Development to date has shown that derivatives of urushiol, the oil in poison ivy that causes dermatitis, to be effective in animal models for desensitization to poison ivy dermatitis and to have desirable bioavailability and toxicological properties. This product has been given "investigational new drug" (IND) status by the Food and Drug Administration. (NP306, C2, PS2a, Project No. 6060-41000-012-00D)

Economically fermenting xylose into ethanol. Xylose is cheap, abundant sugar naturally present in biobased materials, and for the past 30 years scientists have been working to develop brewer's yeast that ferments this sugar into ethanol. Although the process has been successful, the rate of fermentation is still low by industrial standards. To speed up the fermentation process xylose needs to gain faster access into the yeast cells, which occurs via transporter proteins. ARS researchers in Peoria, IL, have developed a yeast that has two novel xylose transporter proteins, thus improving the rate of fermentation rate by 10 percent. Improving the rate of fermentation allows an ethanol plant that produces 40 million gallons per year to produce 4 million extra gallons, bringing down the cost of ethanol production. Increasing fuel ethanol production directly benefits farmers by creating demand for their unused agricultural residues through more efficient biorefining. (NP306, C3, PS3a, Project No. 5010-41000-178-00D)

A novel "green" process for germinated, brown rice beverage. The functional beverage market offers a consumer-friendly mechanism to rapidly develop healthy alternates to dairy products. ARS researchers in New Orleans, LA, developed methods to deliver superior all-natural value-added rice beverages using green technologies. Green technologies for food processing were defined as being sustainable and less harmful to the environment, and entails the use of safe, natural chemical processes to transform raw products into value-added foods and ingredients. The researchers described a novel method for germinating and processing unstabilized raw brown rice that leads to a completely green process and free-flowing soluble matrix that renders a beverage. The "green" sprouted brown rice beverage has no additives, fortifications, added oils, or salts. The method has very low inputs, requires minimal equipment, and is applicable for both germinated brown and colored rice varieties. An invention disclosure was submitted, resulting in the transfer of this knowledge through publication. The global market for plant-based alternatives to dairy beverages is expected to surpass \$34 billion by 2024. This new plant-based, protein-rich functional beverage with proven health-beneficial attributes will have a positive economic impact on that market. (NP306, C1, PS1a, Project No. 6054-41000-107-00D)

Stopping the degradation of sugarcane during temporary storage. Microbial deterioration of sugarcane starts as soon as it is harvested. Harvested cane is commonly stored while awaiting processing, but significant sugar losses occur during storage because of microbial degradation. ARS researchers in New Orleans, LA, working with a private company tested liquid sodium permanganate application to the sugarcane during storage and compared its ability to prevent sugar loss via microbial degradation against other currently used products (bleach, biocide). Sodium permanganate was better than bleach at preventing sugar loss to microbial degradation. A preliminary economic analysis estimates an annual revenue increase for the average Louisiana sugarcane factory to be approximately \$1.95 million using sodium permanganate. Expanded to the entire Louisiana sugar industry, this early

treatment system could increase the annual revenue of the industry by a net of 1.64 percent or \$44.4 million. (NP306, C1, PS1b, Project No. 6054-41000-111-00D)

A new starter culture accelerates the conversion of sugars to acids in the fermentation of commercial pickle production. Out of 243 bacterial isolates from commercial cucumber fermentations, ARS scientists in Raleigh, NC, identified 18 cultures that were able to ferment cucumbers under various conditions of temperature, pH, and salt content. Cultures that were unable to produce potentially carcinogenic biogenic amines in cucumber fermentation brines were selected for further study. These starter cultures produced exopolysaccharides (sugar chains that serve as prebiotics) that feed helpful bacteria that are naturally present in the human gut. Five of the 18 cultures were found to be suitable for use as starter cultures in low-salt fermentations currently undergoing commercial development. One starter culture was transferred to the private sector for commercial production and was used in the United States and in Germany, Mexico, and Turkey for the manufacture of cucumber pickles. The availability of these starter cultures enables the continued and expanded use of the technology to manufacture pickles with low salt and minimized environmental impact. (NP306, C1, PS1b, Project No. 6070-41000-008-00D)

Stevia has no negative effect on the gut. Stevia is a popular plant-based low-calorie sweetener loved by consumers who consume a low-carbohydrate diet. Stevia is a natural plant-based sweetener extracted from the leaves of the *Stevia rebaudiana* plant using water or ethanol, and then dried. Recently, questions have been raised about the safety of stevia and similar extracts. ARS scientist in Wyndmoor, PA, studied the effect of stevia on models of human gut bacteria and found that stevia has no adverse impact on the bacteria in the human gut models. (NP306, C1, PS1b, Project No. 8072-41000-102-00D)

Stopping the yellowing of wool. Wool is a major textile fiber obtained from agricultural animals. However, the long-term exposure of wool to sunlight often causes yellowing and reduces its commercial value. ARS scientists in Wyndmoor, PA, have developed a simple treatment that decreases yellowing by treating wool using a vitamin-like substance, para-aminobenzoic acid (PABA), in combination with high-absorbance ultraviolet (UV) light. The yellowing rate as measured using an absorption-metric colorimeter was greatly diminished when wool fabrics were treated with PABA and UV light. An invention disclosure patent has been submitted and ARS scientists are seeking an industrial partner to commercialize this new process to preserve wool. (NP306, C2, PS2a, Project No. 8072-41440-024-00D)

Improved packaging film made from renewable, inexpensive cotton waste materials. There is increasing concern about the environmental impact of nondegradable plastics accumulating in landfills and the oceans. One solution is to replace some of these fossil-fuel based nondegradable plastics with water-soluble, biodegradable plastics. Poly(vinyl alcohol) (PVOH), derived from plant sources, is water-soluble and biodegradable and the perfect candidate for this replacement. ARS scientists in Peoria, IL, discovered that when PVOH is blended with cotton gin trash (CGT, a waste material left over after cotton processing), it resulted in a low-cost biodegradable composite that is applicable for making composite films or plastics. When CGT is chemically modified, properties of the blended films were further improved for diverse applications. Using CGT in a higher-value product, like biodegradable plastics, increases the value of U.S. cotton coproducts, which benefits U.S. cotton farmers and helps mitigate plastic pollution. (NP306, C2, PS2a, Project No. 5010-41000-174-00D)

Green technique for producing cellulosic composites with improved structural properties. Wood is mainly composed of cellulose and lignin. To remove the lignin is a complex and expensive process that destroys the inherent structure of the wood. ARS scientists in Peoria, IL, have developed a unique green

method for removing lignin from woody biomass that leaves the woody cellulosic structure intact. The removal of lignin from woody biomass is accomplished by using acetic acid and hydrogen peroxide. Novel composites can then be produced by infusing the lignin-free cellulosic with biobased polymers (such as corn starch or corn-derived polylactic acid), which have improved properties. A variety of cellulosic-based materials have been treated in this fashion, including pine and oak. Non-woody samples, such as soy hulls or alfalfa, can also be processed to provide cellulose composites. The wood composites produced using materials from corn will benefit corn growers and producers as well as tree processors by providing another product line for corn and woody products. (NP306, C2, PS2a, Project No. 5010-41000-174-00D)

New renewable, plant-based engine oil additive. There is a great demand in the United States and the world to find new biobased engine additives to help improve lubricant issues found in fossil fuel-based oils. The purpose of an oil is to provide lubrication between two moving metal surfaces. The oil must be of low viscosity (water-like) to penetrate the contact areas yet be viscous enough to provide separation between the moving surfaces at all operating temperatures. As oil heats up in an engine, its viscosity changes and good separation of the moving parts is no longer maintained. ARS scientists in Peoria, IL, used ARS-created and commercialized estolides (made from sunflower and soybean oils) to develop a new type of engine oil additive that solves these engine lubrication problems. When small amounts of these materials are added to an engine oil, the oil's viscosity remains nearly constant over a broad range of temperatures. These new materials are beneficial to farmers, consumers, and retailers because they are environmentally friendly, improve utilization of soybean and sunflower production, and enhance economic security for rural communities. (NP306, C3, PS3b, Project No. 5010-41000-171-00D)

Novel high-value sorghum coproducts. An economic obstacle to producing cellulosic biofuels is the low market price of bioethanol relative to its operating and very high capital costs. ARS researchers in Peoria, IL, working with University of Illinois scientists in Urbana, solved this problem by developing a new process that allows glucose and xylose to be extracted separately from sorghum grain. Glucose is conveniently fermented to ethanol. The xylose syrup was concentrated and then converted to a nutritional product using an engineered brewer's yeast from the ARS microbial collection. The product was beta-carotene, which our bodies use to produce vitamin A. This research will be of interest to those who work on commercializing cellulosic biofuels and more generally to agriculturally-based refiners and farmers looking to find new markets for their crops. (NP306, C3, PS3a, Project No. 5010-41000-176-00D)

New biorefinery-compatible oily yeast strain platform for synthetic biology. Over one billion tons of cellulosic biomass (the structural portion of plants), which can be converted into biofuels and biobased products, can be available throughout the United States without impinging on corn and other row crop production. ARS researchers in Peoria, IL, identified a yeast named *Candida phangngensis* that is suitable for commercial development, closely related to existing commercial yeast strains used in synthetic biology, and generally regarded as safe. The new yeast strain produces a plant-like oil from cellulosic sugars and can be transformed to grow on all biomass sugars to produce even more oil. This new strain is expected to advance the economic feasibility of high-quality biodiesel and jet fuels from renewable herbaceous and woody biomass, reducing U.S. dependence on fossil fuels while supporting rural economies and conserving the environment. (NP306, C3, PS3a, Project No. 5010-41000-177-00D)

**Identification of genes for degrading plant biomass.** Renewable conversion of plants to products begins with breaking down the fibers in plant material. In nature, fungi do the heavy lifting in biomass

breakdown. ARS scientists in Peoria, IL, collaborated with the Joint Genome Institute and scientists from Colombia and the Netherlands to decode the genome of a unique fungus for that natural process. This is the first in-depth genome/transcriptome analysis in an understudied group of fungi with powerful machinery for breaking down plant fibers. More than 50 new genes related to biomass breakdown were identified. This fungus has an arsenal of genes and enzymes needed to break down biomass fibers. The genes and enzymes can be "borrowed" to break down plant polymers and make value-added fuels and chemicals. This research and these results will benefit producers of renewable products who seek effective enzymes to deconstruct the fibers in plant biomass. (NP306, C3, PS3a, Project No. 5010-41000-178-00D)

Use of nano-proteins to provide an antifogging surface. Antifogging agents are chemicals that prevent the condensation of water in the form of small droplets on a surface. Without antifogging treatment, condensed water forms fog-like droplets on the surface of glass or plastics and scatters light, causing low visibility. ARS scientists in Peoria, IL, have improved on a previously patented ARS invention to produce protein nanoparticles that outperform commercial antifog solutions. These biodegradable nanoparticles are made using proteins (from wheat, soybean, or milk) combined with a petroleum-based material. Any surface that may have water fogging or beading on it will benefit. This includes surfaces such as windows on cars, boats, homes, and buildings. In addition, eyewear, such as eyeglasses, and medical and swim googles, will also benefit. End consumers will benefit, and depending on the protein used, so will farmers and stakeholders in the wheat, soybean, and milk value chains. (NP306, C3, PS3a, Project No. 5010-44000-053-00D)

**Improved products using nanocellulose derived from corn stover.** Cellulose is the substance that is responsible for a plant's strength. If cellulose is reduced to a nano scale (one billionth of a meter) it is

called nanocellulose (NC). NC produced from corn stover was developed by ARS scientists in Peoria, IL. NC has been shown to provide value in many end-uses and products, including polymer blends, medical devices, cosmetics, and waste treatment. In all these applications, the NC must be pumped from one location to the next. For the full value of corn stover NC to be realized, its flow properties must be understood. The scientists used state-of-the-art techniques to determine the flow properties of NC suspensions, which will allow for the production of improved NC-based products. Corn producers and processors will benefit from developing a high-value use from what is normally left over on the corn field. (NP306, C3, PS 3a, Project No. 5010-44000-053-00D)

New analytical techniques to characterize silver nanoparticle-treated textiles. Silver nanoparticles are added to clothing for their powerful ability to kill bacteria and fungi and the odors they cause. To develop safe and reliable nanoparticle-enhanced products, it is essential to have proper analytical techniques that evaluate the resulting technologies and products. However, currently available techniques are complicated, destructive, expensive, and time consuming. ARS researchers in New Orleans, LA, developed two simple, cost-effective, fast, and accurate surface-enhanced Raman spectroscopic methods to quantify silver nanoparticles. The first method, which uses plasmonic hot spots of aggregated nanoparticles, measures silver nanoparticles in a (washing) solution. The second method, which uses a dye to generate a distinctive Raman signal, measures silver nanoparticles in a solid (textile) material. These methods are extremely sensitive, accurate, and better than conventional methods in distinguishing silver nanoparticles from other silver species and mapping the distribution of nanoparticles in textiles. ARS researchers verified the uniformity of the developed silver nanoparticleembedded cottons and their washing durability. The first method was supported by the ARS Innovation Fund and the National Science Foundation, and its novelty was highlighted as a featured article on the front cover of the journal Analytical Methods. (NP306, C2, PS2a, Project No. 6054-41000-106-00D)



## ANIMAL PRODUCTION AND PROTECTION

## **National Programs:**

- Food Animal Production, NP 101
- Animal Health, NP 103
- Veterinary, Medical, and Urban Entomology, NP 104
- Aquaculture, NP 106

A high-quality cattle gene atlas. The goal of genomic analyses in livestock is to make sense of the genome to understand and improve important livestock traits. With modern technologies, it is possible to localize variation in traits to regions of the genome, but it can be difficult to determine the gene, and the change in the gene, responsible for the trait. For some genes where the functions are not well characterized, it can also be difficult to determine what parts of the body are involved in controlling the trait. ARS scientists in Beltsville, MD, developed a comprehensive tissue-gene atlas for cattle by integrating their own information with publicly available information on gene regions associated with traits, genes expressed by tissues, and changes in gene chromosome structure that are known to control gene function. This high-quality cattle gene atlas links these three data sources for the first time and provides an important tool for discovering the tissues, genes, and genome structure that control traits in cattle. (NP101, C2, PS2A, Project No. 8042-31000-001-00D)

Chromatin modification in placenta of swine varies with season. Chromatin modifications are known to alter gene expression, and therefore contribute to changes in traits like fertility. In pigs, pregnancy rates are lower when breeding take place during the summer, but the reasons are not known. The swine industry controls temperature and photoperiod by using climate-controlled housing, but seasonal reductions in farrowing rates and litter size still occur. Producers offset these losses by increasing their summer breeding stock 20–25 percent and subsequently increasing the number of breedings by 15 percent, all at significant cost to the industry. ARS scientists in Clay Center, NE, and University of Wisconsin collaborators found that placentas developed from summer breeding had greater expression of genes associated with chromatin modification than placentas from winter breeding. These data suggest seasonal differences in the expression of chromatin modification genes may contribute to seasonal infertility by altering the expression of key genes. Treatments that reduce unfavorable placental chromatin modification pathways during the summer could reduce seasonal reproductive inefficiency losses estimated at more than \$600 million annually. (NP101, C1, PS1A, Project No. 3040-31000-095-00D)

Development of 3-dimensional (3D) imaging to predict the weight of pigs. Continuously monitoring animal weight would benefit producers by ensuring all animals are gaining weight as expected and would assist in precision feeding of pigs. ARS scientists in Clay Center, NE, conducted research on commercially available 3D imaging to predict live animal weight in grow-finish pigs. They collected 920 3D images and weights from a population of grow-to-finishing pigs equally divided by sex across 3 commercial lines. The 3D images were used to calculate the volume of each pig, and scientists developed an equation to predict pig weight from the calculated volumes regardless of sire line or sex. Methods for monitoring the growth and physical condition of animals without animal handling will

reduce labor, improve animal well-being, and increase the profitability and sustainability of production. (NP101, C1, PS1C, Project No. 3040-31000-097-00D)

Development of a verified algorithm to correctly assign sequencing level genotypes to crossbred cattle using low-cost, low-coverage genotypes. Detecting all the DNA variation in each individual animal can be done by genome sequencing, but is too expensive for routine genomic analyses of traits. The full DNA variation of individuals is necessary to effectively predict trait differences caused by DNA variation. Methods to correctly assign complete sequence-level DNA variation of key ancestors to offspring using low-cost genotyping would improve genomic predictions and save industry the expense of sequencing every animal. ARS researchers in Clay Center, NE, assembled genomic sequences from individuals with many descendants in a crossbred population representing the eighteen most predominant beef breeds in the United States, and combined it with publicly available sequences representing beef and dairy breeds. In collaboration with Gencove, Inc., these sequences were analyzed to determine DNA variants that typically occurred together. Those relationships were then used to predict all the variants in the genome from a low-cost, low-coverage genome sequencing to generate a low number of initial genotypes. The low- cost sequencing approach for generating initial genotypes results in better accuracy of DNA variation assignment than genotypes obtained using SNP chips, the most common approach to genetic marker detection. This new method will enable more effective traitgenome associations at a lower cost than currently available SNP chips. Producers will include a larger portion of animals, at low cost, for genetic evaluation programs, and predict genotypes up to the genome sequence level. This will improve selection accuracy and increase genetic gain, and lead to a faster rate of improvement in valuable beef traits for the industry. (NP101, C2, PS2A, Project No. 3040-31000-100-00D)

Classification of beef carcasses for top sirloin tenderness. Recently developed certification standards for tenderness have given the beef industry added impetus to implement a tenderness-based marketing system. For retailers to effectively execute a tenderness-based marketing strategy, retailers must be able to market all meat derived from the loin and rib from a qualifying carcass as certified tender. Currently, the certification does not include top sirloins, which are derived from the loin and represent a substantial retail meat cut feature. ARS scientists in Clay Center, NE, determined that tenderness classes based on the VBG2000 beef grading camera allowed for identification of carcasses with more favorable top sirloin tenderness. This work showed that tenderness testing with a beef grading camera in combination with refrigerated aging for 28 days can produce consistently tender top sirloin steaks that qualify for a guaranteed tender marketing claim. This could lead to more than \$18,000,000 in added annual revenue for the U.S. beef industry. (NP101, C3, PS3A, Project No. 3040-31430-006-00D)

Using nitrous oxide (laughing gas) to euthanize piglets is as effective as using carbon dioxide and may be more humane. A significant component of livestock care is determining when to euthanize hurt or sick animals that will not be able to recover and how this should be conducted to prevent further suffering or injury. Previous research that assessed physiological stress reactions in piglets indicated euthanasia using nitrous oxide instead of carbon dioxide could be less stressful. ARS scientists in West Lafayette, IN, followed up on these findings with additional research on the potential of using nitrous oxide to euthanize piglets that are seriously ill, injured, or unable to thrive because of congenital issues or other factors. Based on several observations, including EEG readings that assessed brain wave activity, they concluded that nitrous oxide may be a suitable alternative to carbon dioxide and a more humane alternative for pigs that must be euthanized. (NP101, C1, PS1C, Project No. 5020-32000-013-00D)

Grazing red clover reverses fescue toxicosis in cattle. Fescue toxicosis, characterized by reduced blood circulation, affects cattle, sheep, and goats in large sections of the Mid-Atlantic region of the United States. It is caused by toxins produced by a fungus infesting fescue grass common in regional pastures. The pasture legume red clover contains isoflavones that can improve blood flow in animals affected by fescue toxicosis. Interseeding red clover into toxic tall fescue pastures is suggested to mitigate fescue toxicosis, but the direct impact of interseeding red clover on blood flow and performance has not been assessed. ARS scientists in Lexington, KY, evaluated the impact of interseeding red clover on cattle grazing toxic tall fescue. Cattle grazing pastures with interseeded red clover had greater growth performance and exhibited increased blood flow post-grazing. This research showed interseeding red clover in toxic tall fescue pastures can reverse some of the negative effects associated with fescue toxicosis. Notably, this is the first study that demonstrated cattle grazing interseeded red clover is an effective pasture management strategy to elicit the benefits of isoflavones on blood flow. (NP101, C1, PS1A and PS1C, Project No. 5042-32630-003-00D)

Stored livestock germplasm is a valuable resource for livestock producers. The livestock industry relies on the National Animal Germplasm Program (NAGP) collection to provide historical samples of genetic diversity and increasingly uses the collection to address threats such as disease and shrinking genetic diversity. To support this critical food security need in the future, ARS scientists in Fort Collins, CO, continue to add to the animal germplasm collection, which in FY 2020 reached the threshold of more than 1 million samples, and greatly expanded its diversity. Demonstrating its use, Brangus breeders found a mutation that causes white eye disease. The NAGP collection contained the only sampling of an important key ancestor (born in 1982) that tested negative for the condition. Genomic testing can be used to eliminate such mutations, but comprehensive testing is cost-prohibitive. Testing key sires can indicate that their offspring do not need testing if the sire and dam are negative for the

disease. The NAGP sample of a key sire reduced the number of cattle tested by 150,000 animals and saved the industry about \$1.5 million. In a second example, the nation's largest seller of beef bulls reintroduced 1980s–1990s genetics to restore genetic diversity to their breeding program. This breeder accessed the NAGP collection for bulls born in 1987 and 1993 with the desired genetics and produced approximately 70 embryos that were implanted in cows that will soon calve. These recent examples highlight the industry value of the NAGP with its diverse genetic collection to address current threats. (NP101, C2, PS2C, Project No. 3012-31000-006-00D)

New method to improve the identification of genetic markers for feed efficiency. Typical studies to identify genetic markers for livestock traits include several thousand animals and thousands of genetic markers. Genotyping, analysis, and interpretation costs associated with the large resulting data set is a challenge to producers wanting to implement genomic selection. To reduce genomic selection's cost and complexity, an improved ability to narrow genotyping to the most appropriate markers to test for association with specific traits is needed. Using tissues relevant to digestive processes from high and low feed efficient pigs, ARS scientists in Clay Center, NE, developed a methodology that used gene expression data to rank the likelihood that the genes contribute to feed efficiency. To provide a reduced set of markers for analysis, scientists selected 10 different groups of genes and markers for separate trait association analyses based on different relationships to criteria related to feed efficiency. Scientists found 36 markers were associated with feed efficiency; 29 were linked with genes already known to have associations with feed efficiency, demonstrating prescreening helps select for relevant genes and markers. A less targeted approach using thousands of genes and markers evaluated many markers, most of which had no influence on the trait. Though they did raise the threshold for statistical significance, the analysis resulted in identifying only two markers associated with feed efficiency. Neither of those markers were in genomic regions previously associated with feed efficiency and, despite using stringent

criteria, may be spurious. The novel strategy for using gene expression information to preselect markers for genomic analysis is a powerful approach for identifying economically important livestock traits markers. The markers identified in this study are available to commercial genotyping companies for producers to improve pig feed efficiency. (NP101, C2, PS2A, Project No. 3040-31000-097-00D)

Optimal donor age for transplantation of ovarian tissue in the turkey. For most livestock species, frozen sperm effectively captures the entire genome because sperm contains both sex chromosomes (X and Y). However, in birds the sex chromosomes are W and Z and males are ZZ. Sperm from male birds only contains a single sex chromosome, so frozen sperm does not preserve the unique W female sex chromosome. An alternative approach to the preservation of the entire genome in birds is to freeze immature ovarian tissue for storage, and then thaw and transfer it into recipients. Stem cells in the transplanted ovarian tissue then undergoes meiosis to generate eggs derived from the tissue donor. ARS scientists in Beltsville, MD, in collaboration with scientists in Canada, took a step toward developing methods to preserve and transfer ovarian tissues in turkeys. Using fresh ovarian tissue, they determined the optimal age to recover and transplant ovarian tissue is 7 days post-hatch; 91 percent of grafts attach and grow if ovarian tissue from 7-day old donors is transplanted into 2-day old recipients. This result represents a major advancement in preservation of turkey germplasm, because turkeys are an agriculturally important species with historically poor sperm cryopreservation success. (NP101, C2, PS20, Project No. 2018 8042-31000-110-00D)

Genome sequencing of the weaning-associated fungus *Kazachstania slooffiae* to understand its potential role in piglet growth and health. Weaning is a period of stress and environmental change for piglets, and they experience a greater incidence of diarrhea and other digestive problems. Recent studies indicate there are dramatic changes in fungal microorganisms after weaning, and this change may

contribute to the growth and health of weaned piglets. ARS scientists in Beltsville, MD, isolated *Kazachstania slooffiae*, the most dominant post-weaning fungus in healthy piglets, and sequenced its genome. Genes identified from this sequence suggested that *K. slooffiae* has positive interactions with beneficial bacteria in the piglet gut, signifying a strong beneficial role. Sequencing this genome is a critical first step for investigating the effects of this microbe in piglet growth and health. These results support the concept that *K. slooffiae* can be used as a naturally derived probiotic to enhance piglet growth. (NP101, C1, PS1A and PS1C, Project No. 8042-31440-001-00D)

## Development of a safe and effective African swine fever virus vaccine -ASFV-G-delta I177L.

African swine fever (ASF) is a devastating and highly lethal disease of pigs for which there are no commercial vaccines. One of the most significant knowledge gaps that has hindered scientists from developing a safe and effective ASF vaccine is the lack of genomics information on the function of the virus's 150 genes. ARS scientists at Orient Point, NY, successfully developed genetic engineering techniques that systematically delete genes from the ASF viral genome to determine their function. Pathogenicity studies in pigs with these altered viruses led to the discovery of essential genes for ASF viral replication, host immune evasion, and determinants of virulence. Gene identification provides potential targets for a rational design of safe and efficacious gene-deleted vaccines. The most recent vaccine candidate is the discovery of a genetically engineered gene-deleted live attenuated vaccine strain called ASFV-G-delta I177L. This vaccine strain was shown to be safe and effective and exceeded the performance of other ASF vaccine candidates. For the first time, the ASFV-G-delta I177L vaccine was shown to fully protect pigs against ASF with a low dose of vaccine virus. The safety characteristics of the vaccine include no adverse events even when high doses of the vaccine were administered to pigs. A patent covering the development ASFV-G-delta I177L was filed and several commercial partners initiated the process of licensing ASFV-G-delta I177L. ARS scientists have established an agreement

with one of these companies to initiate the commercial development of the vaccine. (NP103, C1, PS1a, Project No. 8064-32000-060-00D)

Improved computer modeling to predict susceptibility of different species to infection with severe acute respiratory coronavirus 2 (SARS-CoV-2). Viruses need to enter a cell to replicate and cause infection. Viral entry begins with attachment between a virus protein and a cell receptor(s), which allows the virus to enter the cell. Once inside the cell, the virus initiates replication and starts the race between host immunity and infection. For coronaviruses, the spike protein on the viral surface is responsible for cell receptor binding and cell entry. Several groups report that SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE2) as the primary receptor for cell attachment. The susceptibility of both wild and domestic animals to SARS-CoV-2 and the potential for its zoonotic transmission is a public health concern. Assessing the potential for zoonotic transmission includes: (1) screening to identify the animal species that was infected originally and passed SARS-CoV-2 to humans; (2) determining if animal hosts can amplify SARS-CoV-2, which could increase its infectious potential; and (3) determining the current risk of infected people passing the virus to animals, particularly domestic species. The latter could result in an amplifying zoonotic cycle of human to animal to human transmission that could worsen SARS-CoV-2 evolution and prevalence. ARS researchers in Ames, IA, evaluated cross-species ACE2 genetic diversity in expression and functionality to determine susceptible tissue types and susceptibility of different animal species to SARS-CoV-2. The analysis predicted the limited potential of livestock transmission of SARS-CoV-2. Results also revealed that evolutionary changes in the genetic sequence of ACE2 receptors in domestic animals, including dogs, pigs, cattle, and goats, may have resulted in restricting SARS-CoV-2 infections. (NP103, C1, PS1b, Project No. 5030-32000-118-00D)

A reference typing method and related public database for Mycoplasma bovis. Mycoplasma bovis is a bacterial pathogen that causes significant respiratory disease in cattle and bison. An objective, standardized, and discriminatory method to categorize bacterial isolates is needed to understand how this bacterium spreads and whether particular families of isolates have an enhanced ability to cause disease. Two different genetic typing methods have recently been developed for M. bovis, but the related data available fail to reveal whether one is superior to the other. To resolve this issue, a subcommittee of the International Organization for Mycoplasmology requested ARS researchers in Ames, IA, to organize and lead a multinational effort to compare the two methods and identify a single approach as a universal typing scheme. ARS scientists defined a single, highly informative method employed as the reference typing scheme for M. bovis using bioinformatics tools to analyze genome sequences from more than 450 isolates obtained from every major region of the world. The scheme and a related open-access, curated database are freely available online at pubmlst.org/mbovis. The database integrates genetic data with isolate-specific information, such as geographic and anatomic origin, year of origin, clinical presentation of the animal of origin, and other factors. This comprehensive resource currently includes more than 1,200 isolates and has been accessed by more than 100 animal health researchers and clinicians around the world. Information from the database was a critical part of several recent studies that defined local, regional, and global transmission patterns of M. bovis. Such insights into the population structure and epidemiology of M. bovis will support the development of rational, data-driven management and treatment practices that will positively impact livestock farmers and consumers of related products. (NP103, C4, PS4a, Project No. 5030-32000-116-00D)

Validation of an international standard PPD (purified protein derivative) for skin testing cattle.

Bovine tuberculosis, caused by the bacterium *Mycobacterium bovis*, is a global problem impacting international and domestic trade. Harmonization and acceptance of diagnostic tests for bovine

tuberculosis are important production and trade issues. ARS scientists in Ames, IA, worked internationally with other bovine tuberculosis research labs and the World Organization for Animal Health to evaluate and validate a new international standard tuberculin, a sterile protein extract *of M. bovis* that is used for skin testing cattle for bovine tuberculosis. This new standard creates a global organized system to ensure uniform testing worldwide. These findings will benefit regulatory agencies, veterinarians, and livestock producers involved with maintaining the tuberculosis-free status for the United States. (NP103, C3, PS3c, Project No. 5030-32000-222-00D)

Venereal transmission of vesicular stomatitis virus in biting midges. Biting midges are well-known agricultural pests that transmit vesicular stomatitis virus (VSV) to cattle, horses, and swine. Vesicular stomatitis outbreaks occur every 3–8 years in the United States and result in significant economic losses due to animal disease, animal movement restrictions, and quarantines. In temperate regions, viruses appear to overwinter in the absence of infected animals through unknown mechanisms, resulting in new infections the following year. ARS scientists in Manhattan, KS, collaborated with Kansas State University to examine whether VSV could pass between male and female midges during mating to better understand whether virus may be maintained in insect populations in multi-year outbreaks. They found that during mating, VSV-infected females could transmit virus to uninfected naïve males that had never been exposed to the virus, and that infected males could transmit virus to uninfected naïve females. This research shows the importance of males in VSV transmission dynamics, and the role vectors may play in the maintenance of VSV. This is the first evidence for venereal transmission of any arbovirus (viruses that infect arthropods) in biting midges, and the first evidence for venereal transmission of VSV in any known vector species. These results highlight the need to incorporate alternative routes of transmission in understanding arbovirus outbreaks, and could lead to a more comprehensive understanding of: 1) potential virus persistence in nature between outbreaks; 2) the

ability of some virus strains to survive through the winter leading to multi-year outbreaks; and 3) virus transmission dynamics during VSV outbreaks. (NP103, C1, PS1b, Project No. 3020-32000-013-00D)

Duration of contagion of foot-and-mouth disease virus in infected live pigs and carcasses. Datadriven modeling of high-consequence foreign animal disease outbreaks is a critical component of veterinary preparedness. However, research and data are needed to address knowledge gaps in disease dynamics and modeling. ARS scientists in Orient Point, NY, conducted a study to address two major gaps in knowledge of foot-and-mouth disease virus (FMDV) pathogenesis in pigs: 1) the duration of the infectious period; and 2) the viability of FMDV in decaying carcasses. ARS scientists demonstrated that infected pigs transmitted disease at 10 days post infection (dpi), but not at 15 dpi. Assuming a latent period of 1 day, this results in a conservative estimate of 9 days infectious duration, which is considerably longer than suggested by previous research conducted with cattle. The residual airborne contagion diminished within 2 days of removal of infected pigs from isolation rooms. Furthermore, FMDV in muscle was inactivated within 7 days in carcasses stored at 4°C. By contrast, FMDV infectivity in vesicles harvested from intact carcasses stored under similar conditions remained remarkably high until the study termination at 11 weeks post-mortem. This information may be used to update models used for FMD outbreak simulations involving areas of substantial pig production and emphasizes that infected carcasses can be a potential source of virus contamination and must be handled properly to prevent FMDV transmission. (NP103, C1, C1a, Project No. 8064-32000-061-00D)

**Performance of a brucellosis diagnostic test in domestic and wildlife species.** Billions of dollars have been invested at State and Federal levels to eradicate brucellosis from the United States. Brucellosis is a bacterial disease largely controlled in domesticated livestock, but it has been very difficult to eradicate because of reinfection from wildlife reservoirs such as bison, elk, and feral swine. It is a zoonotic

disease that can spread from cattle to people and cause severe human illness. The fluorescence polarization assay (FPA) is a highly sensitive and specific test commonly used for brucellosis surveillance under field conditions. Although designed for use in cattle, the test is also used for surveillance in elk, swine, and bison using negative control cattle sera included in the kit. ARS scientists in Ames, IA, tested sera from non-infected swine, bison, and elk against sera from control, vaccinated, and Brucella-infected animals to determine if species differences could influence responses to the test. The data demonstrate the assay results are influenced by the species of background sera, which is important for surveillance studies and regulatory agencies. Some species' sera were incorrectly interpreted as positive for brucellosis. Additionally, the assay does not perform as well in swine and did not detect animals persistently infected with the *Brucella abortus* strain RB51 vaccine. This work will be of interest to regulatory personnel in states with brucellosis-infected wildlife, those conducting surveillance studies, and livestock producers. (NP103, C3, PS3a, Project No. 5030-32000-224-00D)

A computational method to quantify the effects of genome replication errors on bacterial evolution. Bovine respiratory disease costs the industry hundreds of millions of dollars each year through treatment costs and losses in production. Bovine respiratory disease is often caused by a complex of viruses and bacteria. Researchers do not understand how some bacteria normally found in the respiratory tract can suddenly become virulent. ARS researchers in Clay Center, NE, developed a novel method to study how bacterial respiratory pathogens such as Mannhemia haemolytica, Histophilus somni, and Bibersteinia trehalosi suddenly become virulent. These bacteria all possess genomic features consisting of a single base or multiple bases, repeated in series, called simple sequence repeats (SSRs). These SSRs allow the bacterial genes to change rapidly through replication errors that occur during DNA synthesis. Sometimes the change is great and alters the appearance, virulence, or pathogenicity of the bacterium and is referred to as phase variation. Developing methodology to track these replication

errors will greatly increase the ability to understand how different bacteria cause disease and advance the development of mitigation strategies. (NP103, C4, PS4a, Project No. 3040-32000-034-00D)

Effect of environmental temperature on the ability of mosquitoes to transmit Rift Valley fever virus. ARS researchers in Manhattan, KS, and collaborators at the U.S. Army Medical Research Institute for Infectious Diseases evaluated how environmental temperature affects the ability of two mosquito species native to the United States to transmit Rift Valley fever virus (RVFV). Mosquito incubation temperatures ranging from 14°C to 26°C were evaluated for their effect on virus infection, dissemination, and transmission rates. For both mosquito species tested, increased temperature was associated with more rapid and more efficient infections and increased the potential for mosquitoes to transmit RVFV. Although environmental temperature affected the ability of RVFV to replicate and amplify in mosquitoes, the effect differed between the two species. The results indicated that the effect of temperature on RVFV dissemination and transmission was species dependent. These data on the effects of ambient temperature facilitate development of more accurate models to assess RVFV persistence and spread in nature should a disease outbreak ever occur in the United States. (NP103, C1, PS1b, Project No. 3020-32000-009-00D)

Characterization of a new strain of bovine viral diarrhea virus isolated in the United States. A new bovine viral diarrhea virus (BVDV) strain was isolated in California. While belonging to the BVDV1 grouping of BVDV viruses, it does not belong to the BVDV1a or BVDV1b groups that are known to be in the United States. Characterizing this virus is important to determine the effectiveness of current bovine vaccines to protect against emerging BVDV viruses. ARS scientists in Ames, IA, in collaboration with researchers at the University of California, Davis, revealed this virus was a novel BVDV1 strain, now called BVDV1i, that had not been previously reported in the United States. Genetic

analysis of the virus sequence showed this virus had only been reported in Europe and South America. Further tests were conducted using antibodies raised against BVDV strains found in commercial bovine vaccines and results indicated these antibodies recognized the BVDV1i strain but at a reduced level, so current vaccines may only provide partial protection against emerging BVDV1. (NP103, C4, PS4a, Project No. 5030-32000-117-00D)

The first U.S. national survey of *Mycoplasma ovis* in domestic sheep operations. *Mycoplasma ovis* is a blood pathogen in sheep, goats, and deer. While its presence has been documented in the United States, there had been no large-scale study documenting its prevalence and geographic range among sheep. ARS researchers in Pullman, WA, in collaboration with researchers at the USDA Animal and Plant Health Inspection Service, performed the first national survey of *M. ovis* in U.S. sheep. Samples were collected from more than 34,000 U.S. sheep as part of the National Animal Health Monitoring Surveys, and *M. ovis* was present in 73.3 percent of U.S. sheep operations. Furthermore, when present, *M. ovis* occurred in 23.3 percent of sampled individuals within the flock. These data demonstrate *M. ovis* is more common than previously believed in the United States and may contribute to anemia, jaundice, and ill-thrift (failure to thrive) for many sheep. (NP103, C4, PS4d, Project No. 2090-32000-036-00D)

Genomic regions of *Streptococcus suis* reveal differences that may contribute to the spectrum of clinical disease. Different bacterial isolates of *S. suis* cause a spectrum of disease in pigs which complicates studies to understand how the bacterium causes disease. ARS researchers at Ames, IA, evaluated the ability of nine isolates of *S. suis* to cause disease following intranasal challenge in swine. This was followed by comparative genomic analyses to identify genetic differences in the isolates potentially associated with swine virulence. Challenge outcomes ranged from lethal systemic disease to no signs of disease. Whole genome sequencing followed by comparative genomic analyses revealed

several notable regions of difference, including regions encoding secreted and membrane-associated factors, which likely contributed to the spectrum of clinical disease observed. In addition, transmissible elements containing antimicrobial resistance genes were identified within the *S. suis* genomes.

Collectively, these results provide a foundation for understanding the genomic attributes responsible for the spectrum of virulence that exists among *S. suis* isolates. This information is essential for designing effective vaccines needed by the swine industry to mitigate *S. suis* disease and reduce public health concerns. (NP 103, C4, PS4b, Project No. 5030-32000-119-00D)

Attractant-impregnated sticky film for stable fly mass trapping. Stable flies feed on livestock and cause significant animal stress and loss of vigor. This leads to production losses, increased susceptibility to disease, and sometimes death. They are also a nuisance pest of domestic pets, zoo animals, and humans in some U.S. coastal areas. Current stable fly control methods are ineffective and too costly. ARS scientists in Lincoln, NE, in conjunction with an industry partner, developed an attractant-impregnated adhesive tape for mass trapping stable flies. The traps can reduce stable fly induced stress and defensive behaviors of confined cattle by up to 80 percent, and result in improved cattle weight gain and milk production. This technology also has potential for reducing stable flies on pets, zoo animals, and on humans in coastal communities. An international patent was granted on this technology. (NP104, C1, PS1B, Project No. 3042-32000-011-00D)

Treated military uniform compromised by pesticide-resistant mosquitoes. Military camouflage uniforms are routinely treated with permethrin to repel disease-carrying mosquitoes. This preserves an effective fighting force while military members operate in environments where mosquito-vectored diseases are endemic. ARS researchers in Gainesville, FL, and U.S. Department of Defense collaborating scientists completed and published a study clearly demonstrating that treated military

uniform efficacy may be completely compromised by pyrethroid-resistant strains of *Aedes aegypti*, a vector of numerous human diseases. Fortunately, this study also showed that the protection provided by N,N-Diethyl-meta-toluamide (DEET)-based repellents was unaffected by resistance to pyrethroids. This information will aid in the risk assessment of different operational environments and lead to changes in procedures and chemicals used to protect at-risk military members operating in areas where pyrethoid-resistant mosquitoes exist. (NP104, C2, PS2A, Project No. 6036-32000-052-00D)

Discovery of synergists for topical and spatial repellents that circumvent pesticide resistant mosquitoes. Pyrethroids are one of the most commonly used classes of insecticides. The acids of three common pyrethroids were shown to be nontoxic to mosquitoes. ARS researchers in Gainesville, FL, along with partners at the University of Florida, discovered these pyrethroid acids have significant synergistic spatial repellent activity. Synergists are compounds that, when added to a pesticide or repellent, greatly increase the efficacy or repellency over the pesticide or repellent alone. The repellency measured was greater than the topical repellent N,N-Diethyl-meta-toluamide (DEET), was often better than the parent pyrethroids, and showed little cross resistance in a pyrethroid-resistant Puerto Rico strain of mosquitoes. Most important, synergism caused by the pyrethroid acids, when combined with several repellent compounds, increased protection of human arms from biting mosquitoes. Using these synergists may increase bite protection for existing and novel compounds, including protection against resistant mosquitoes that can transmit diseases such as dengue, Zika, chikungunya, and yellow fever. (NP104, C2, PS2A, Project No. 6036-32000-052-00D)

House flies collected in agricultural settings carry antimicrobial-resistant bacteria. House flies are primary pests of confined livestock operations such as dairies; due to their close associations with humans, they are nuisance pests in domestic settings. Adult flies frequent microbe-rich substrates such

as garbage dumpsters and animal manure, where they encounter and ingest bacteria during feeding and reproduction. To assess human and animal health risks, bacteria were enumerated from whole flies, identified to species, and tested for susceptibility to 14 antimicrobials. Both male and female flies carried antimicrobial-resistant (AMR) bacteria: 36 of 38 isolates (95 percent) were resistant to more than one antimicrobial, 33 were multidrug-resistant (MDR); and 24 isolates were resistant to more than 4 antimicrobials. These results emphasize the role flies may play in harboring and disseminating bacteria, including AMR and MDR strains and potential pathogens that pose a risk to human and animal health. The results add to the growing evidence implicating flies as major players in disease ecology, epidemiology, and the dispersal of AMR genes. (NP104, C1, PS1C, Project No. 3020-32000-018-00D)

**House flies carry and can potentially transmit bacterial pathogens associated with bovine respiratory disease.** House flies are major nuisance pests at feedlots and are of concern to animal health since they acquire, harbor, and transmit numerous pathogens. Bovine respiratory disease (BRD) is an economically important and complex illness of cattle associated with several bacterial and viral species. It is not clear what role flies play in harboring and transmitting bacterial pathogens associated with BRD. ARS scientists in Manhattan, KS, collected house flies from a commercial feedlot where cattle were suffering from apparent respiratory illness. Two different methods were used to examine the prevalence of the three main BRD bacterial pathogens *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* in male and female flies. Using both methods, *M. haemolytica* was detected in 11.7 percent of house flies, followed by *P. multocida* (5 percent) and *H. somni* (3.3 percent). The presence of BRD bacterial pathogens in house flies suggests they can play a role as reservoirs and disseminators of the bacteria in the feedlot environment. Further, infected flies pose a risk transmitting BRD when they acquire pathogens from sick animals and subsequently associate with healthy animals. (NP104, C1, PS1C, Project No. 3020-32000-018-00D)

Novel insecticide shows promise for fly pests of livestock. Heavy reliance on chemical control resulted in widespread resistance to almost all available marketed pesticides for filth and biting flies.

ARS researchers in Gainesville, FL, and Kerrville, TX, and researchers at Northern Illinois University, The Pennsylvania State University, and Cornell University evaluated the effectiveness of the novel insecticide fluralaner against house flies and horn flies resistant to two commonly used insecticides, imidacloprid and permethrin. When fed to flies in sugar bait, fluralaner was 23-fold more toxic to a susceptible house fly strain than imidacloprid and more than 117-fold more toxic to an imidacloprid-resistant house fly strain. It also out-performed permethrin when applied directly to the flies. At present, fluralaner is only registered for use on dogs and cats for flea control. These results indicate this material would be very useful against insecticide-resistant flies. (NP104, C1, PS1C, Project No. 6036-32000-053-00D and 3094-32000-041-00D)

Toxicity of chitosan for house flies, horse flies, and blow flies. House flies, horse flies, and blow flies are important pests of humans and their associated animals. ARS scientists in Gainesville, FL, and researchers at the University of Massachusetts examined insecticidal properties of chitosan, a polysaccharide derived from chitin. Chitosan fed to adult house flies (*Musca domestica*), horse flies (*Tabanus nigrovittatus*), and blow flies (*Phormia regina*), was found to be toxic to all three. Chitosan appears to disrupt the microorganisms that live in the gut of the fly. It is easily made, commercially produced, non-toxic to mammals, biodegradable, and used in a range of agricultural applications. Chitosan shows promise as a new environmentally friendly pesticide against filth and biting flies affecting humans and livestock. (NP104, C1, PS1C, Project No. 6036-32000-053-00D)

Natural compounds as insecticides and fly repellents. Fly resistance to chemical pesticides is an increasing problem and highlights the need to develop alternatives to existing fly control chemical pesticides. ARS scientists in Kerrville, TX, demonstrated the effectiveness of a number of natural compounds, including essential oils and other botanical compounds, as repellents or insecticides in laboratory bioassays against various life stages of horn flies. Cinnamon oil, spearmint oil, citronellol, and limonene each exhibited repellent activity comparable or greater than N, N-Diethyl-meta-toluamide (DEET) to horn flies. Unlike DEET, these natural plant compounds also exhibited insecticidal activity against horn flies. These compounds can be incorporated into integrated pest management strategies to suppress horn fly populations associated with livestock production, reducing losses to livestock producers and potential annoyance to nearby human populations. (NP104, C1, PS1C, Project No. 3094-32000-041-00D)

Virus infection makes flies lose their appetite. Previous studies have shown that Musca domestica salivary gland hypertrophy virus (MdSGHV) dramatically enlarges salivary glands and prevents or delays ovarian development in its adult host, the common housefly, and could potentially be used as a biocontrol method. The effect of the virus on the fly's natural food consumption, however, remained unexplored prior to this study, which was conducted by ARS scientists in Gainesville, FL, and researchers at the University of Massachusetts. Both virus-infected and control flies were provided a choice of an 8 percent sucrose solution or a 4 percent powdered milk solution to determine food preferences. Healthy females with developing ovaries continued to consume a sugar and protein diet while infected females fed predominantly on a sugar diet. Infected flies of both sexes consumed less food than healthy flies. Infected flies in the field may spend less time visiting food sources, which could reduce their survival and the risk of human pathogen movement. (NP104, C1, PS1C, Project No. 6036-32000-053-00D)

Beauveria bassiana is only effective against house fly larvae during a brief time window. Using entomopathogenic fungi such as Beauveria bassiana to manage adult house fly populations shows promising results, but little is known about whether it can be used against house fly larvae. ARS researchers in Gainesville, FL, demonstrated that temperature and diet did not modify the effectiveness of B. bassiana treatments against fly larvae. Additional testing revealed fly larvae are only susceptible to the pathogen when they are very young, and very high fungal doses are required to kill them. The results show that B. bassiana can control adult flies effectively but that larval control with this biocontrol agent is prohibitively expensive. (NP104, C1, PS1C, Project No. 6036-32000-053-00D)

Natural spread of a virus in imported fire ants. Introduced from South America, the red imported fire ant currently infests more than 128 million hectares of land in the United States and is estimated to cause damage exceeding \$7 billion annually. *Solenopsis invicta* virus 3 (SINV-3) is an RNA virus specific for red imported fire ants that offers promise as a natural control agent. It infects queens and immature ants and results in a colony weight reduction of more than 50 percent. ARS scientists in Gainesville, FL, and researchers at Florida A&M University conducted surveys to determine the prevalence of SINV-3 in winged female fire ants to understand the possible natural spread of the virus through mating flights. Collections were made from five urban areas and five adjacent north Florida rural areas. SINV-3 was detected in winged females in nests from 7 of the 10 collection locations. The average infection rate of 44 percent was similar in rural and urban areas. Winged females were sampled because they mate aerially and disperse, founding colonies in new areas. Infected winged females may be the mechanism of SINV-3 spread throughout the fire ant community and may provide additional sustained control of fire ants in the United States. (NP104, C3, PS3C, Project No. 6036-32000-051-00D)

A super colony of invasive ants in Florida. The tawny crazy ant is an invasive ant from South America that infests Florida and Texas and is spreading to states along the Gulf Coast. Extremely large populations of this ant inundate urban and natural landscapes, resulting in mass intrusions into buildings as well as reductions in ant biodiversity. ARS researchers in Gainesville, FL, determined that tawny crazy ants did not fight with other tawny crazy ants from different nests located at the same site, or with ants from nests located as far as 270 miles away. In fact, small fragments of colonies from distant nests, including queens, congregated together in the same nests in laboratory tests. Tawny crazy ants are not territorial over large areas. These findings suggest that tawny crazy ants in Florida are part of a super colony across the southern United States and that the lack of territorial behavior facilitates resource sharing and the movement of worker ants and brood between colonies. These characteristics could be used in developing control strategies, including the spread of natural enemies such as pathogens and toxic baits being developed for their control. (NP104, C3, PS3B, Project No. 20196036-32000-051-00D)

Development of a rapid resistance assay to aid in mosquito control. Pyrethroids have been used in the control of mosquitoes for years. Due to their heavy use, many populations of mosquitoes became resistant to various pyrethroids. Rapid detection of resistance allows for the proper pesticide selection for mosquito abatement. ARS researchers in Gainesville, FL, and collaborating scientists at the U.S. Department of Defense developed a novel, rapid assay for assessment of pyrethroid resistance in the southern house mosquito, *Culex quinquefasciatus*. This assay is quick, inexpensive, and fits within existing assay systems for several species of mosquitoes. The assay is currently being tested in a Florida study and will soon be implemented in Louisiana. (NP104, C2, PS2A, Project No. 6036-32000-052-00D)

New and quick method to identify fever ticks resistant to pyrethroids. Cattle fever ticks were eradicated from the southeastern United States but continue to reinfest parts of southern Texas. The presence of wildlife complicates eradication efforts by spreading ticks across the Mexico-United States border into Texas. Pyrethroids are a class of pesticide used to control tick infestations on cattle in Mexico and on U.S. wildlife. However, many tick populations in Mexico are resistant to pyrethroids. Quick and accurate diagnosis of pyrethroid resistance in the cattle fever tick is critical for selecting the appropriate pesticide to use. ARS scientists in Kerrville and Edinburg, TX, and Pullman, WA, worked with collaborators at Northern Arizona University and the University of Queretaro (Mexico) to develop a new and quick method to identify fever ticks resistant to pyrethroids. This assay can detect multiple changes in the gene coding for the protein targeted by pyrethroids and which can lead to resistance. This molecular assay can be completed overnight while the traditional bioassay method takes 6 weeks to complete. The ability to quickly detect pyrethroid-resistant ticks allows decision makers to choose the proper pesticide to use on wildlife during Texas outbreaks. (NP104, C1, PS1A, Project No. 3094-32000-042-00D)

Release of the Delta Select strain of channel catfish. Improved catfish germplasm in aquaculture will reduce production costs and allow U.S. catfish farmers to remain competitive in the global seafood market. ARS researchers in Stoneville, MS, developed the 'Delta Select' strain of channel catfish through three generations of genetic selection, leading to a 25 percent increase in growth rate and 0.9 percent increase in carcass yield compared to the non-selected Delta Control line that originated from the same population. Since fillet yield is somewhere around 45%, changes of even 1% (of the 350 million pounds of fish harvested annually) would result in a big difference (3.5 million pounds). Approximately 90,000 head (180,000 pounds) of 2-year-old Delta Select catfish were released to

industry, providing U.S. farmers access to improved catfish germplasm that will make them more efficient and profitable. (NP106, C1 PS1B, Project No. 6066-31000-016-00D)

Genome-enabled breeding tools for Atlantic salmon. The number of U.S. commercial Atlantic salmon farming operations is expected to increase 5-fold over the next 3 years, and demand for genetically improved stocks will increase dramatically. ARS researchers in Franklin, ME, and Leetown, WV, created an improved genome reference sequence for the North American Atlantic salmon and developed the first DNA chip that enables the use of genomic information in breeding strategies. This DNA chip is publicly available and in use by commercial breeding programs. (NP106, C2, PS2A, Project No. 8030-31000-005-00D)

A rapid assay for *Flavobacterium columnare*. Columnaris disease is caused by the bacterium *Flavobacterium columnare* and negatively impacts almost all finfish aquaculture industries in the United States and worldwide. Previous research established the existence of four distinct genetic groups within the species *F. columnare*; however, there were no quick and easy methods to assign an unknown isolate to one of the four groups. Knowing which group is causing outbreaks informs decisions on how to respond. ARS researchers in Auburn, AL, developed a molecular assay to quickly assign an isolate to a genetic group, demonstrating the assay is rapid, sensitive, and specific for genotyping *F. columnare*. The assay is inexpensive and can be used by any laboratory with basic molecular capabilities to determine the genetic group(s) responsible for disease outbreaks. It is currently used by commercial and academic laboratories. (NP106, C1 PS1B, Project No. 6010-32000-027-00D)

An alternative aquafeed protein source improves growth and immune responses. Frass is a byproduct of the fly larval meal industry and is composed of larval excrement, shed exoskeletons, and

residual fly feed. ARS researchers in Auburn, AL, evaluated diets containing black soldier fly frass as partial replacements for soybean meal, wheat short (the fine bran particles, germ, and particles separated in commercial flour milling), and corn meal. Experimental diets were fed to fingerling catfish and tilapia and the results demonstrated that final weight gain was significantly increased. Additionally, tilapia fed frass diets exhibited increased survival against two important pathogens, *Flavobacterium columnare* and *Streptococcus iniae*. Based on these findings, frass derived from the larvae of black solder flies has potential as an alternative source of protein in aquafeeds or as an ingredient enhancing palatability and growth. (NP106, C1, PS1A, Project No. 6010-32000-027-00D)

Pyranopyrans are potential bacteriocidal compounds against fish pathogens. Disease losses in catfish aquaculture can cost up to \$100 million, so reducing or eliminating bacterial pathogens is critical to the success of the industry and improving fish health and welfare. ARS researchers in Oxford, MS; Stoneville, MS; and Villanova University modified the chemical structures of natural compounds produced by a certain species of fungus to produce novel compounds. The novel compounds pyranopyrans possess significant antibacterial activities against certain species of fish pathogens. Combatting disease with alternative efficacious natural or natural-based compounds is very acceptable to the catfish industry, which has access to only a few approved therapeutants and is striving to limit the use of antibiotics. (NP106, C1, PS1B, Project No. 6066-31000-016-00D)

A bacteriophage for preventing disease in rainbow trout. Bacteriophages (phages) are viruses that infect and kill bacteria, self-replicating in high numbers in the process. Used against disease-causing microbes, phages are excellent candidates for the prevention or treatment of bacterial diseases. ARS researchers in Leetown, WV, identified a new phage that kills *Yersinia ruckeri*, the rainbow trout pathogen. This phage is unique; in addition to killing its bacterial host by infection, it also binds to and

degrades lipopolysaccharide, a large carbohydrate structure that covers the surface of some bacteria and reduces the effectiveness of the trout immune system. By trimming off this protective layer, the phage renders *Yersinia ruckeri* susceptible to the trout immune system, preventing its survival inside its fish host. (NP106, C2, PS2B, Project No. 8082-32000-007-00D)

Improved method to measure starch content and gelatinization. Starch is an important component of various food and feed products. Starch gelatinization (DGS) is important during processing starch-containing foods or feeds; the degree of DSG affects physiochemical and sensory properties of starchy products and their susceptibility to enzymatic digestion and so affects their nutritional availability for humans or animals. ARS researchers in Hagerman, ID, recently developed an improved method for simpler and more accurate measurement of total starch and gelatinized starch in situ for wet and dried products. This provides a valuable new tool for food and feed researchers to study the role of starch in food and feed products. (NP106, C2, PS2A, Project No. 2050-21310-006-00D)

A draft genome sequence for Florida pompano. The lack of available genome information is a hurdle in implementing state-of-the-art selective breeding strategies for many aquaculture species, including Florida pompano. ARS-funded researchers in Fort Pierce, FL, established a complete draft genome of the Florida pompano by using a hybrid sequencing method and a novel bioinformatics workflow. This draft genome will improve farm production and profitability and enhance breeding strategies by identifying genes associated with aquaculture production efficiency and product quality. (NP106, C5, PS5A, Project No. 6034-22000-044-00D)

Catfish diets and feeding strategies affect meat yield. During the production and harvest cycle, catfish farmers are sometimes unable to sell and must hold market weight catfish for processing if there is a

lack of demand. Feeding fish during this holding time allows the fish to grow larger than the preferred size for processing. If feed is withheld during this time, the fish mobilize muscle tissue for energy, reducing fillet yield. In either case, the price paid to farmers is reduced. ARS researchers in Stoneville, MS, worked in cooperation with Mississippi State University fish nutritionists and determined that hybrid catfish fed once or twice weekly for 4 months remained in the preferred size range for processing but had reduced fillet yield. Thirty days of full feeding were needed to restore fillet yield to normal levels in fish fed once or twice weekly for 4 months. These results allow the catfish farming industry to develop feeding strategies that minimize production costs and maximize profits. (NP106, C1, PS1B, Project No. 6066-31000-016-00D)

Nutritional requirements of Florida pompano broodstock. Quality broodstock diets increase reproductive success and seedstock quality, leading to increased hatchery success, on-farm efficiencies, and profitability. The lack of optimal diets for Florida pompano broodstock (especially during the spawning season) continues to present an obstacle to commercial production, because nutritional status is a powerful determinant of egg quality and the successful development of eggs and larvae. ARS-funded researchers in Fort Pierce, FL, employed comprehensive and quantitative lipid analysis to determine different egg and larval lipid compositions and identify lipid requirements for larval development and successful reproduction of Florida pompano. Hatchery managers and marine finfish producers will benefit from efficiencies associated with meeting optimum nutritional needs for reproduction and successful seedstock production. (NP106, C5, PS5A, Project No. 6034-22000-044-00D)



## NATURAL RESOURCES AND SUSTAINABLE AGRICULTURAL SYSTEMS

#### **National Programs:**

- Water Availability and Watershed Management, NP 211
- Soil and Air, NP 212
- Grass, Forage and Rangeland Systems, NP 215
- Sustainable Agricultural Systems Research, NP 216

A long-term solution for thirsty crops. A cost-effective means of increasing plant-available water can alleviate water stress from infrequent precipitation or limited irrigation supplies. Polymer hydrogels increase the capacity of soil to hold water, but the effects were previously thought to last only a few years. ARS researchers in Kimberly, ID, conducted a 9-year study to measure the effects of a single hydrogel application on plant-available water in soil. Based on the slow decline in water availability seen in this study, the water retention benefits of hydrogels should last from 24 years to 29 years, considerably longer than current industry estimates. The long-term water retention benefits substantially increase the cost effectiveness for farmers applying hydrogels to improve soil's water holding capacity. (NP211, C3, PS B, Project No. 054-13000-009-00D)

**Deficit irrigation saves water in peach production under arid conditions.** Agricultural irrigation is a major user of fresh water in arid and semiarid areas of the world. About 23,000 hectares of peaches

grown in the Central Valley of California depend on irrigation, which uses scarce water resources. Deficit irrigation is a potential strategy to save water without severely impacting crop production; however, the long-term impact of deficit irrigation on productivity is not well understood. ARS researchers in Parlier, CA, demonstrated in a 10-year field study that deficit irrigation can result in up to 40 percent water savings without significant yield losses or reductions in fruit quality such as firmness, total soluble solids, pH, malic acid, or total phenolics. Findings from this long-term research provide peach growers an alternative irrigation strategy to save water and lower input costs. (NP211, C1, PSA, Project No. 2034-13000-012-00D)

Center pivot mounted leaf temperature sensors are inexpensive and provide accurate input to irrigation water optimization. In times of low crop prices, farmers need to produce crops as inexpensively as possible. One way farmers can reduce input costs is to irrigate only when it is most needed. Crop leaf temperatures are easily measured by sensors, which provide a real-time assessment of water stress and in turn indicate if irrigation should be scheduled. However, users of temperature sensors have been concerned that measurements from sensors mounted on a center pivot may not be as accurate as non-moving (stationary) sensors. ARS scientists in Bushland, TX, compared irrigation scheduling based on data from stationary temperature sensors to those mounted on a center pivot. There were no differences in accuracy between stationary or moving temperature sensors, and irrigation application scheduling governed by one type of sensor was similar to scheduling governed by the other. Center pivots are now used on 30 million acres in the United States. Installing temperature sensors aboard center pivots and using them for irrigation scheduling could save farmers substantial water and reduce energy input costs. (NP211, C1, PSA, Project No. 3090-13000-015-00D)

New water budgets across the Long-Term Agroecosystem Research (LTAR) network. Management of intensified agricultural production and climate can affect soil water storage and water movement in agricultural landscapes. Understanding these relationships is critical to more efficient and sustainable water use. ARS scientists in Columbia, MO, along with collaborators at all 18 LTAR sites, have developed agricultural site water budgets that account for all inputs and outputs of water on an average annual basis. The LTAR network covers a wide range of values for yearly precipitation, evaporation, plant water uptake, surface runoff, and subsurface flow. The LTAR modeling group is using these water budgets to validate their models across the network. (NP211, C4, PSA, Project No. 5070-12130-006-00D)

Alternative crops grown in saline soils provide value-added products. Drought-, salt-, and borontolerant plant species that are adapted to grow with high saline drainage or ground waters in poor-quality soils are valuable commodities in the western United States. ARS researchers in Parlier, CA, are testing the viability of several specialty crops in saline drainage sediment and in saline/boron soils in the San Joaquin Valley. They are irrigating salt- and boron-tolerant poplar-tree clones, Opuntia cactus, guayule, mustard, agretti, and pistachio with drainage waters containing high levels of salt, boron, and selenium. All tested plants and trees produced selenium-enriched plant products, and guayule produced increased amounts of latex and resin under saline conditions. The successful use of alternative drought-, salt-, and boron-tolerant crops and the production of new biobased products provide growers new agronomic strategies and alternative crops for continuing production in drainage-impacted regions of the western Central Valley of California. (NP211, C1 PSF, Project No. 2034-13000-012-00D)

Modified blind inlets structure design improves dissolved phosphorus removal. Blind inlets, which are limestone-gravel filters for agricultural drainage water in field depressions, were shown to be

effective at reducing losses of particulate phosphorous (P) (i.e., P bound to sediment) by virtue of sediment filtration. However, typical blind inlets are ineffective at removing dissolved P, which is a greater water quality hazard than particulate P. ARS scientists in West Lafayette, IN, improved a blind inlet by constructing it with steel slag, a material that has a high affinity for dissolved P, and monitoring for impact on water quality. Over a 3-year period, the use of steel slag removed 45 percent of the dissolved P, 18 percent of the organic nitrogen, 67 percent of the ammonium, and 70 percent of the glyphosate and dicamba from field runoff. Steel slag costs for blind inlets are similar to the costs of aggregates and provide a simple update for improving removal of dissolved P and some pesticides. The benefit to growers is that it reduces their negative impact on water quality and increases production sustainability by preventing the loss of nutrients and pesticides. (NP211, C2 PSD, Project No. 5020-12130-003-00D)

Soil and leaf sensors improve irrigation scheduling for water conservation. Because of limited water resources on the Texas High Plains, producers are interested in growing grain sorghum, which requires less water than corn to produce maximum yields. However, precision irrigation scheduling tools are needed to optimize regional sorghum production. ARS scientists in Bushland, TX, in collaboration with the Rural Development Administration of South Korea and University of Nevada at Reno, have used automated irrigation scheduling based on leaf canopy temperature with and without data from soil moisture sensors to manage grain sorghum at high, medium, and low irrigation levels. The scientists found that plant and soil water sensing with multiple stress thresholds and several different irrigation volumes led to the most water efficient irrigation management for grain sorghum producers in Texas.

The methods achieving the highest water use efficiency were readily automated. Irrigators implementing such a system will save time, use less energy, and reduce groundwater withdrawals. (NP211, C1, PSA, Project No. 3090-13000-015-00D)

Sub-surface drip reduces seasonal irrigation applications for corn. In the face of declining water supplies, crop farmers need to maximize the yield per unit of water used in crop production, the so-called crop water productivity (CWP). It is not well understood how irrigation application methods affect CWP. ARS scientists in Bushland, TX, compared the water use and yield of grain corn and sorghum production using sprinkler and subsurface drip irrigation (SDI) methods. Using the SDI application method, loss of water to evaporation was reduced by 2-5 inches during the growing season, compared to losses that occurred with sprinkler irrigation. SDI reduced overall corn water use by up to 6 inches and increased grain yields by up to 20 percent. The combined effects were an increase in CWP by up to 46 percent, compared with sprinkler irrigation. The increases in CWP are enough to offset the higher costs for SDI installation. (NP211, C1, PSC, Project No. 3090-13000-015-00D)

Effective nitrate removal from tile drained fields in Iowa using saturated riparian buffers. The saturated riparian buffer is a conservation practice that diverts agricultural tile drainage into streamside soils, which can effectively remove nitrate from drainage water at little cost. Conservation planners want to understand the potential role of this practice in addressing nitrate losses from agricultural watersheds with tile drained cropland. ARS scientists in Ames, IA, applied the Agricultural Conservation Planning Framework (ACPF) in 32 Iowa watersheds to determine the extent of riparian zones suited for saturated buffers, and the extent of tile drained lands found above those same riparian zones. Riparian lengths suited to the saturated buffer practice occupied 30-70 percent of streambanks in most watersheds and could treat tile drainage from 15 to 40 percent of the watershed areas. Saturated buffers have an important potential role for water quality improvement in many tile drained watersheds in Iowa and to a lesser extent where riparian practice options are limited. These results are useful for conservation

planners seeking to identify viable options to reduce nitrate loads from Midwestern agricultural watersheds. (NP211, C3, PSA, Project No. 5030-13000-011-00D)

Terrestrial sources of urea shown to contribute more than urea fertilizer to toxic algal blooms. Global increases in the frequency and toxicity of algal blooms in coastal waters are raising concerns over agricultural use of urea, the most common form of commercial nitrogen (N) fertilizer. ARS researchers in University Park, PA, with colleagues from the University of Maryland-Eastern Shore and Penn State University, evaluated urea transport in field drainage, runoff, and stream water within an agricultural basin on Maryland's Atlantic Coastal Plain. Results showed that runoff of recently applied urea in early spring is usually diluted below levels of environmental concern. However, in summer, stagnant water in small field ditches and wetlands creates ideal conditions for microbial production of urea that flushes into local streams and subsequently flows to coastal waters. Producers can manage drainage control structures to allow ditches to drain freely during summer. This will minimize the development of stagnant water pools, which will greatly reduce the risk of harmful algal blooms due to urea flushing from agricultural drainage ditches into streams and coastal waters. (NP211, C2, PSA,

Legume cover crop impacts on sugarcane production. Sugarcane is a commercially important crop in Louisiana, Florida, and Texas, and the sugar produced is worth over \$1 billion (U.S.) annually. However, monoculture sugarcane production can degrade soils by reducing soil organic matter and enabling soil pathogenic microorganism proliferation. Cultural practices that improve sugarcane sustainability are thus needed to maintain yields in fields with degraded soils. Legumes can be grown to improve soil health during the normal fallow period between sugarcane crops. ARS scientists in Houma, LA, in collaboration with scientists at Louisiana State University AgCenter and Alma Plantation, completed multi-year and location trials that investigated how sunn hemp and cowpea cover crops

Project No. 8070-13000-014-00D)

affected subsequent sugarcane yields. On average, the cover crops provided 4.3 tons per acre of dry biomass and 200 pounds per acre of nitrogen. Cowpea generally improved subsequent plant cane yields, but the effects of sunn hemp varied. However, neither cowpea nor sunn hemp reduced cane or sucrose yields consistently in subsequent sugarcane crops. Legume cover crops can be an important component of sustainable sugarcane production practices and are now being used by growers in several parishes in Louisiana. (NP211, C4, PSC, Project No. 6052-13210-002-00D)

## Mapping of manure sheds helps improve manure nutrient utilization and the environment.

Nutrient recycling is fundamental to sustainable agricultural systems. Few mechanisms exist, however, to ensure that surplus manure nutrients from confined animal feeding operations (CAFOs) are effectively transported for use in nutrient-deficient croplands. These nutrients sometimes concentrate in locations where they can threaten environmental health and devalue manure as a fertilizer. As part of the Long-Term Agroecosystem Research (LTAR) research effort, an ARS team from multiple locations, led by scientists in Las Cruces, NM, classified the 3,109 counties of the contiguous United States by their capacity to either supply manure phosphorus (P) and nitrogen (N) or assimilate and remove excess P and N via crops ("sinks"). ARS scientists in St. Paul, MN, assisted in the analysis and are co-leading a follow-up effort focusing on the dairy component of manure production and use. Manure sheds—areas surrounding livestock operations where excess manure nutrients can be recycled for agricultural production—differed in the transport distances needed to assimilate excess manure P based upon the type of CAFO (from  $147 \pm 51$  km for a beef-dominated manure shed to  $368 \pm 140$  km for a poultrydominated manure shed). This highlighted the need for systems-level strategies that operate across local, county, regional, and national scales to promote manure nutrient recycling. This LTAR approach is now being applied to each animal segment (dairy, hog, beef, poultry) at the national level to close the loop

between animal manure production and plant nutrient needs, which will reduce contamination of ground and surface waters in animal production regions. (NP211, C2, PSD, Project No. 5062-12130-007-00D)

Semiarid grassland nitrous oxide emissions increase with warmer temperatures. Although much climate change research focuses on carbon dioxide, there are other important greenhouse gases occurring in smaller quantities, but with greater potency than carbon dioxide, including nitrous oxide (N<sub>2</sub>O). ARS researchers in Tucson, AZ, and colleagues from China analyzed 46 published studies worldwide in which temperature or precipitation were artificially altered to test for effects on N<sub>2</sub>O emissions. They found that increased temperature drove increased N<sub>2</sub>O release from soils by an average of 33 percent, although the results varied across biomes, with the biggest response in shrublands. Increased precipitation also enhanced N<sub>2</sub>O emissions, while reduced precipitation suppressed emissions. Collectively, these results suggest that globally warming temperatures may increase N<sub>2</sub>O release, representing a reinforcing effect on climate change in the future. (NP211, C2, PSB, Project No. 2022-13610-012-00D)

Long-term dataset demonstrates value of native cover in protecting water quality. Long-term research is important to understanding how land management affects runoff and erosion in agricultural production systems. ARS scientists have compiled, discussed, and published 23 years of historical data measured in 8 fields located in El Reno, OK. Results indicated that native tallgrass prairie fields had 98 percent, 72 percent, and 78 percent lower suspended sediments, total soluble phosphorus, and nitrate-nitrogen losses, respectively, than cropped fields. This research database is essential for determining the impact of different agricultural management systems, understanding the processes related to hydrologic transport and water quality, and the development and validation of the corresponding models. These data contribute to national initiatives, including the Long-Term Agroecosystem Research network and the

Conservation Effects Assessment Project, that are working to quantify the effects of land management on soil and water resources under variable climate. (NP211, C2, PSA, Project No. 3070-13000-012-00D)

Late planted corn requires less irrigation water. Because of declining water levels in the Southern Ogallala Aquifer region, alternative management strategies are needed to reduce groundwater withdrawals while maintaining profitable crop yields. Delayed planting of corn on the Texas High Plains is believed to reduce irrigation requirements by taking advantage of increased precipitation and reduced evapotranspiration demand. However, limited field data exist for corn planting dates in the region. ARS researchers at Bushland, TX, and Texas A&M AgriLife used a calibrated Soil Water Assessment Tool (SWAT) model with long-term historical climate data to simulate corn irrigation and yields for both long and short season corn varieties. Simulation results suggested that irrigation requirements of short season corn were at least 25 percent lower than requirements for long season corn, while grain yields decreased by less than 9 percent. Data from field experiments conducted in 2016 and 2017 with drought tolerant corn hybrids corroborated these trends. These results indicate that the delayed planting of corn combined with effective irrigation management have the potential to reduce groundwater withdrawals from the Ogallala Aquifer. These results are useful to irrigators as a means of extending their groundwater resources and reducing their energy input costs. (NP211, C1, PSA, Project No. 3090-13000-015-00D)

# Adaptive nutrient management demonstrates enhanced economic and environmental outcomes.

With increasing variability in climatic and economic drivers, producers who have previously used static agricultural management strategies may want to pursue adaptive management principles to improve net returns and potentially other ecosystem services. ARS researchers at Temple, TX, managed cropped fields in the Riesel Watersheds for 16 years using 0-8 tons of poultry litter per acre for their annual

application rates. During this period, management progressed from static management to adaptive management using the Haney Soil Health Test, which reduced nitrogen (N) application rates in fields with poultry litter applications and recommended cover crops. Using adaptive nutrient management reduced N application rates by 25 to 38 percent for low rates of poultry litter without sacrificing profitability. Poultry litter application rates in excess of crop phosphorus demand increased phosphorus runoff losses while reducing profitability. Long-term studies that analyze field-scale agronomic, economic, and environmental factors are extremely rare, since they are expensive and labor intensive. This LTAR study shows producers, conservation professionals, and policy makers the utility of adaptive management principles over the long-term as one potential suite of practices to balance economic and environmental outcomes through agronomic management. (NP211, C4, PSA, Project No. 3098-13610-008-00D)

Method for partitioning deisopropylatrazine in streams. Streams within the Salt River Basin of northeastern Missouri are chronically contaminated with the triazine herbicides atrazine and simazine and their common metabolite deisopropylatrazine (DIA). However, in order to link stream pesticide levels to herbicides applied in the fields, one needs to know the parent source of DIA. Therefore, a method is needed to partition DIA between its two parent sources – i.e., DIA derived from atrazine (DIAATR) and DIA derived from simazine (DIASIM). ARS scientists in Columbia, MO, along with University of Missouri cooperators, developed a method based on the concentration ratios of simazine to atrazine (SAR) in streams. The SAR method performed better than two other methods based on concentrations of chloro-triazines in field runoff. The SAR method results demonstrated the differences in DIASIM and DIAATR transport timing, with peak DIASIM transport occurring from mid-November to April and peak DIAATR transport from May to June. In the Salt River Basin, dual-season triazine applications substantially increased the period of high chloro-triazine concentrations in streams from

approximately 3 to 8 months per year. This new method provides water resources and conservation managers the means to identify the parent herbicide and target conservation efforts toward its management in order to improve water quality. (NP211, C2, PSA, Project No. 5070-12130-006-00D) Assessing RUSLE2 and WEPP differences as a conservation planning tool. To streamline delivery of conservation assistance to farmers, the USDA Natural Resources Conservation Service (NRCS) plans to transition from the Revised Universal Soil Loss Equation 2 (RUSLE2) to the Water Erosion Prediction Project (WEPP) to guide conservation planning regarding erosion by water. However, there are concerns that estimated erosion rates may increase as a result of the transition, thereby adversely impacting farmers' conservation compliance. ARS researchers in Oxford, MS (RUSLE2 Team), and West Lafayette, IN (WEPP Team), conducted almost 40,000 simulations covering different climate, soil, land management, terrain, and crop yield conditions for counties in Illinois and Iowa. The soil loss estimates for about half of the simulation scenarios were statistically different between RUSLE2 and WEPP. In comparable scenarios, the primary differences were related to model soil erodibility characterization, slope length effects, no-till management, and cover crop managements. WEPP was sensitive to the quality of climate inputs, so future work should include comprehensive evaluations of different climate scenarios, as well as data precision, gaps, and resolution. The performed assessment is vital to conservation management planning provided by NRCS and farmer's conservation compliance under specific provisions of the 2018 Farm Bill. (NP211, C3, PSA, Project No. 6060-13000-026-00D)

A new modeling approach for soil and gully erosion research. A new 2D numerical simulation model has been developed by ARS researchers in Oxford, MS, to simulate soil erosion and gully erosion processes in field sized watersheds. This physically based model mimics rainstorm induced watershed runoff, splash erosion, shear erosion of soil, and sediment transport processes in high resolution. The simulation results were validated using experimental and field observation results collected by Federal

agencies. This new capability helps hydrology and agriculture engineers in erosion control research and provides the NRCS a powerful tool (http://websim.rusle2.org), that will help evaluate ephemeral erosion throughout the country. (NP211, C3, PSA, Project No. 6060-13000-028-00D)

Strategic management using new and improved decision tools can improve farm productivity and environmental impacts. Producers and researchers need decision-making tools to estimate how their production decisions influence farm productivity and environmental impacts. ARS researchers in Fort Collins, CO, collaborated with Colorado State University to upgrade the DayCent model, and with American Farmland Trust (AFT) to develop the CaRPE tool. ARS researchers improved DayCent by accounting for soil freeze-thaw effects on nitrous oxide (N2O) emissions, representing soil organic matter dynamics to 30-cm depths, addressing how cover crops and their removal affect greenhouse gas (GHG) emissions, and a range of other management factors. These changes increased the accuracy of GHG emissions modelling, which in turn produced more accurate GHG emissions that were 22 percent higher from 1990 to 2017 relative to the previous inventory. The COMET-Farm tool imbeds algorithms from the DayCent model and is frequently used for field and farm productivity and GHG estimation. It provides much of the modeling power of the CaRPE tool, an interactive management tool that couples crop and grazing land data from the NASS Ag Census with county-level GHG emission reduction coefficients from COMET. CaRPE has been used to explore the regional and national potential for agriculture to reduce GHG emissions and combat climate change, and AFT testified about its impacts before the House Select Committee on the Climate Crisis. These tools will enhance the ability of farmers to manage their systems, provide better estimates of and strategies to control GHG emissions, and provide a better foundation for additional scientific discoveries and agricultural management. The tools are useful to producers, NGOs such as the AFT and The Nature Conservancy, and government

organizations, including NRCS, EPA, and NOAA. (NP212, C1, PS1A, PS1B, C2, PS2A, Project No. 3012-11120-001-00D)

Phenol accumulation in California rice soils correlates with late-season inhibition of crop nitrogen uptake. A key factor of rice grain yield is late-season crop uptake of soil nitrogen (N), which promotes grain filling. Previous ARS research in experimental rice plots in Arkansas indicated that inhibition of late-season crop N uptake was correlated with soil levels of phenol, which at higher levels can inhibit N uptake. Recently, ARS researchers in Ames, IA, collaborated with the University of California, Davis to analyze soil from fields on working farms in the Sacramento Valley that varied widely in late-season rice uptake of N. Soil phenol concentrations were moderately correlated with late-season rice N uptake, providing first-time, on-farm evidence to strengthen the previous ARS findings from Arkansas. Variation in soil phenol content may be caused by differences in how fields are aerated during the season. The basis for increased soil aeration arose partially from ARS rice research in Arkansas, and is now being studied in additional rice-producing states in the United States. The practice of increased soil aeration has also been incorporated into the Sustainable Rice Platform (http://www.sustainablerice.org/), which is a global organization composed of more than 100 institutional members. It is striving to improve rice management practices of one million small farmers worldwide. (NP212, C2, PS2A, Project No. 5030-12000-015-00D)

Precision agriculture has a new tool for creating soil carbon content maps. Soil carbon mapping is extremely useful in assessing how land management practices affect and promote crop productivity, agroecosystem sustainability, and ecosystem services. ARS researchers in Auburn, AL, have developed a unique mobile system that uses neutron-gamma analysis to assess and map soil carbon to a depth of 30 cm (plow layer) in real-time. The team coupled their mobile system to a Global Positioning System

device to simultaneously acquire soil carbon content and geographical positions for field mapping. The reliability of soil carbon measurements was found to align well with traditional soil sampling, well within the 95 percent prediction bands. The approach yields an efficient, geo-referenced tool to facilitate rapid, inexpensive, and accurate construction of soil carbon maps. This methodology is licensed for commercialization and will be useful to all crop producers, the national and global soil health community, such as NRCS and the UN FAO Global Soil Partnership, and university researchers and NGOs. It has potential applications for other soil assessment needs in the areas of construction and land restoration for the BLM and even the DOD. (NP212, C1, PS1A, PS1B, Project No. 6010-11120-008-00D)

Standardizing soil analysis provides an understanding of the soil microbiome and its relationship to soil health. Soil health is a complex concept that requires fundamental characterization of the living soil ecosystem. There is a critical need for reliable and consistent scientific methods (such as microbiome analysis that depends on advanced DNA methods) to do that characterization. Standardizing protocols for sample collection, sample handling, laboratory analysis, and data collection and analysis is critical to reduce study variability, improve interpretation of results, and increase the use of soil health assessments for developing better soil health management for productivity, sustainability, or resilience to climate change. A consortium of ARS scientists in Pendleton, OR; Fort Collins, CO; Brooking, SD; Riverside, CA; Columbia, MO; Fayetteville, AR; Florence, SC; Beltsville, MD; Lincoln, NE; Stoneville, MS; and Morris, MN, developed such a protocol to address sources of variability and uncertainty in measuring microbial community composition and its connection with agricultural management and changing climate. Success in these areas is essential for deriving a "return on investment" for managers considering a shift to soil health-promoting practices. The tools and data are important to the soil health community in the United States, the global community (e.g., the UN FAO Global Soil Partnership), and

researchers from public and private institutions worldwide who are interested in applying information on the genetic potential of soil to enhance soil ecosystem sustainability. (NP212, C1, PS1A, PS1B, PS1C, Project No. 0500-00034-001-00D)

New agriculturally focused collection of E. coli types for enhanced animal and food safety.

Escherichia coli (E. coli) is a bacterial species commonly isolated from humans and animals as an indicator of fecal contamination of water supplies and environments, and more than 700 types (e.g., serotypes) of E. coli have been identified. Although most E. coli serotypes found in animal production are harmless, they are shown to be a source and reservoir for antibiotic resistance genes that may be transferred to animal or human pathogens. This antibiotic resistance is a significant concern for the health of producers, their animals, and the public at large, so understanding their differences will help in identification and mitigation strategies. Most E. coli collections focus on human health, and very few collections represent the genetic variability and virulence of those affecting U.S. food animal production. ARS researchers in Florence, SC, led the assembly of AgEc, a publicly available collection that identified and studied 300 E. coli variants found in four major animal production commodities – beef, dairy, poultry, and swine. This was a collaborative effort of 15 researchers from 9 ARS research units, universities, and NRCS partners, and addressed production systems across 12 states. The team tested the collection serotypes for resistance genes to two common antibiotics, tetracycline and sulfonamide, and analysis of antibiotic resistance gene patterns revealed significant differences along commodity and geographical lines. This AgEc effort provides a new publicly available database useful to producers and food- and animal-inspection communities about the types and distribution of E. coli antibiotic resistance in animal production farms. It will assist in determining sources of fecal contaminants in water systems, food, and environments. (NP212, C3, PS3A, Project No. 6082-12630-001-00D)

Stacking management practices improves soil health. Linking specific management practices to measurable changes in soil properties is a key objective for increasing adoption of those practices to improve soil health. ARS scientists in Brookings, SD, used two long-term field studies to show how adding or stacking new practices to base management can affect soil structure and microbial activity. Both studies had base management that included no-till. In one study, crop rotation diversity was added as a new practice, and in the second study, crop residue retention and cover cropping were added. Changes in the extent to which added managements changed soil properties often depended on at what point samples were taken during the crop rotation, showing that stacking management is not simply additive. However, no negative effects were observed from stacking soil health building practices to the no-till crop systems. Implementing multiple positive management tactics increases the likelihood that improvements in soil properties related to better health can be measured. This information is valuable to scientists researching related topics, extension personnel advising producers, and producers selecting management tactics as part of their strategy to increase their ecosystem services. (NP212, C1, PS1B, Project No. 3080-12620-005-00D)

Groundwater quality in Upper Midwest is affected by human and dairy pollutants. The groundwater aquifer in northeast Wisconsin is vulnerable to contamination because it has thin overlying soils that allow contaminants from the land surface to move rapidly to the water table. Because of these conditions, wastewater from septic systems and livestock manure from farming operations are possible contaminant sources for nearby wells. ARS researchers in Marshfield, WI, found up to 28 percent of private wells in Kewaunee County, WI, had detectable coliform bacteria (an indication of fecal contamination) or nitrate-N concentrations greater than 10 mg/L. Furthermore, pathogens that can cause human illness were found in private wells and both human wastewater and cattle manure were identified as sources of the fecal contamination in the contaminated wells. The researchers also tested 964 samples

from 145 wells supplying public water systems in Minnesota and found that fecal contamination was present in 58 percent of samples and 96 percent of wells. They also found that simple tests used by public water systems for bacteria indicative of fecal contamination were good at predicting pathogen absence, but not pathogen presence, and would not be reliable for estimating illnesses from drinking fecal-contaminated groundwater. These findings are providing opportunities for communities in the region to understand the extent of water contamination by human and animal pathogens and for improving groundwater quality for residents. (NP212, C3, PS3A, Project No. 5090-12630-005-00D)

New poultry manure amendments reduce environmental impacts. Ammonia gas emissions from poultry houses and phosphorus (P) loss in runoff from fields where litter (manure + bedding) is applied are two environmental problems from poultry production. Because spreading alum in poultry houses reduces both these problems and improves bird health, the practice is used for about 40 percent of the broiler chickens in the United States. Recently, however, adding alum has not reliably reduced littersoluble P as much as in the past. In fact, adding alum increased soluble P in litter that had been treated with sodium bisulfate, a popular ammonia-control product. ARS researchers in Fayetteville, AR, discovered that adding small amounts of calcium-based nanoparticles to litter treated with alum and/or poultry litter treatment caused a synergistic reaction that resulted in very low soluble P. The researchers also found that treating litter with alum mud, which is a byproduct remaining after alum manufacture, reduced ammonia emissions from poultry litter an average of 40 percent, which was not significantly different from the 35 percent reduction in emissions from alum alone. This presents a sustainable use for the alum mud byproduct, which is currently being landfilled at a cost of \$32 per ton. These combined practices of using alum, nanoparticles, and alum mud could be applied to 80 percent of broiler production facilities in the United States to improve bird health and reduce environmental impacts. (NP212 C3 PS3B Project # 6022-63000-005-00D)

Enhancing crop yields with beneficial crop symbionts. ARS researchers in Wyndmoor, PA, developed molecular tools to identify, quantify, and characterize 11 species of agriculturally relevant arbuscular mycorrhizal fungi (AMF) to enable the selection of the most beneficial AMF for crops. This team previously developed methods for on-farm production of mixed-species AMF inoculum, which, when applied to seedlings, increased yield of several crops (including strawberries, tomatoes, eggplant, peppers, potatoes, and leeks). However, it was not possible to measure or determine how AMF affected individual species. The team then developed quantitative PCR (qPCR) detection assays for 11 different species of AMF found in agricultural soils. These assays identified and quantified the fungi directly from soil samples and in plant root tissues, enabling spore enumeration from soil samples within a single workday, compared to the previous assessment approach that took more than a month to complete. The qPCR method enables direct measurement of individual AMF species colonization efficiency on plant roots, which was not previously possible, and provides an indication of which AMF species are likely to have the most beneficial impacts on crop productivity. These advanced tools will improve agricultural sustainability by guiding the selection of AMF species for propagation and development as AMF biofertilizers—products that improve nutrient acquisition and water uptake and reduce susceptibility to pathogens and disease. (NP212, C1, PS1A, PS1B, PS1C; C2, PS2A, PS2B, Project No. 8072-12000-013-00D)

New method rapidly quantifies field spatial overlaps and gaps for precision agriculture tools.

Auto-guided tractors can reduce on-farm inputs by as much as 20 percent and nationally save producers \$10.8-13.5 million each year by improving equipment efficiency and enhancing yields. Lowering fertilizer and herbicide applications can reduce potential losses to the environment. About half of large-scale crop producers are using tractor guidance, but 82 percent of U.S. farms are small farms that are

largely not adopting these cost-saving and environmentally sound technologies. ARS researchers in Fayetteville and Booneville, AR, in collaboration with the University of Arkansas, developed a novel automated method to rapidly determine spatial overlaps (up to 6 percent of the total field area) and gaps (up to 16 percent of field area) in machinery coverage, and how much precision technologies, such as auto-guided tractors and other self-propelled machinery, can reduce those overlaps and gaps. Tractor guidance systems during field operations improve the average overall efficiency by 8 percent, reducing input use and in-field operation time. This novel approach of estimating tractor guidance efficiency on small farms can aid in adoption of tractor guidance, potentially improving efficiency gains on 82 percent of U.S. farms. (NP212, C3, PS3C, Project No. 6022-63000-005-00D

New economical and efficient strategies remove antibiotics from wastewater and increase agricultural safety and human health. Understanding the removal mechanism of antibiotic compounds and antibiotic resistance determinants in agricultural systems is a global challenge that is important in the protection of human health. An ARS researcher from Riverside, CA, and collaborator from University of California, Riverside, designed and tested a layered environmental media system (consisting of gravel, sand, soil, and a "soil plus biochar" combination) through which antibiotic-laden water was pumped. Overall removal efficiencies of four tested antibiotics—amoxicillin, cefalexin, sulfadiazine, and tetracycline—were 81, 91, 51, and 98 percent, respectively. If the exposure time was lengthened, the removal efficiency was increased, especially for amoxicillin and cefalexin. Overall, the results from this lab-scale proof of concept system indicate the potential of the system for antibiotic removal from wastewater, and highlight ways in which removal efficacy improvements may be scalable for broad applicability. The results of this study will be used by wastewater treatment facilities, the World Health Organization, researchers, and other local municipalities in many developing countries. (NP212, C3, PS3A, Project No. 2036-32000-005-00D)

New research targets less enteric methane emissions from cattle. Cattle produce the greenhouse gas methane as a natural byproduct of digestion, and researchers are investigating ways to help reduce these enteric methane emissions. ARS scientists from Bushland, TX; Woodward, OK; and El Reno, OK, in collaboration with Texas A&M AgriLife Research, studied how cattle methane emissions were affected by hay nutritional quality and fiber content. Cattle fed a high-quality hay diet (high crude protein) produced less methane per unit of digested organic matter than when fed low-quality hay. ARS researchers at Bushland, TX, and Ames, IA, also showed that adding tannin-rich peanut skin, a common regional byproduct, to cattle diets can suppress rumen microbial methanogenesis. This research shows scientists and producers the multiple benefits of feeding higher quality hay to improve animal performance and reduce emissions and indicates the feed additives that may provide cost-effective mitigation of enteric methane from beef and dairy cattle. (NP212, C3, PS3B, Project No. 3090-31630-005-00D

Conservation tillage rebuilds surface soil organic carbon and nitrogen content. Conservation tillage is an effective management strategy to rebuild soil organic carbon and total nitrogen levels. However, there are few long-term studies available to quantify either the rate of change or measurable benefits.

ARS researchers in Florence, SC, created a long-term tillage and crop management experiment using sandy soils. Topsoil samples were collected annually over 37 years under conservation and conventional tillage, and organic carbon and total nitrogen content were measured. Topsoil under conservation tillage had 23 percent more organic carbon and 16 percent more total nitrogen than topsoil under conventional tillage. Furthermore, both tillage systems seem to have reached a new equilibrium where total organic carbon is no longer increasing. These unique results obtained over nearly 4 decades will help landowners and greenhouse gas technical assistance providers determine the climate value and amount

of carbon credits from implementing conservation tillage practices under new USDA carbon credit programs. (NP212, C1, PS1B, Project No. 6082-12630-001-00D)

Pulverized wastepaper helps rehabilitate military training lands. The United States Army produces a significant amount of classified paper waste that is pulverized to a fine consistency unsuitable for recycling. However, this cheap, high quality organic material can be useful as a soil amendment. ARS researchers in Auburn, AL, worked with the U.S. Army Corps of Engineers to develop technology that uses pulverized wastepaper to rehabilitate military training lands. Application of paper waste to soils had no adverse environmental effects, improved soil physiochemical properties, and improved the establishment of desirable native vegetation. When combining cost savings associated with landfill disposal of the paper with savings from greater land rehabilitation success, every ton of diverted paper saves an estimated \$300. At the recommended application rate, this results in a cost savings of approximately \$4,700 per acre. At the installation level, this equates to an estimated annual costs savings of \$20,000 and the diversion of 70 tons of paper. (NP212, C3, PS3D, Project No. 6010-11120-008-00D)

Strategies to reduce the causes of nitrous oxide emissions from beef cattle feed yards. Nitrous oxide is a potent greenhouse gas linked to climate change. Elevated nutrient concentrations make livestock manure a source for nitrous oxide production. Scientists from Bushland, TX; Clay Center, NE; and Texas A&M AgriLife Research investigated how weather affected nitrous oxide emissions from commercial beef cattle feed yards. They determined that nitrous oxide emissions were greatest under warm temperatures and shortly after rainfall events. The team determined that producers and regulatory personnel could manage these emissions more effectively when they can better couple weather patterns with animal agriculture's weather-mediated nitrous oxide emissions. The researchers also developed an empirical model to assess the effect of manure removal frequency on annual nitrous oxide emissions as

related to these conditions. These data and tools in development will be useful for updating national greenhouse gas emissions inventories for beef cattle feed yards. In addition, the cost of managing onfarm nutrients can be improved for increased manure value, which is an important bottom line for producers. (NP212, C2, PS2A, C3, PS3B, Project No. 3090-31630-005-00D)

# Alfalfa leaf protein concentrate is a sustainable alternative to fish meal for aquaculture diets.

Aquaculture is the production of fish and shellfish in controlled conditions and is the fastest growing food sector worldwide. However, fishmeal used in diets of most aquaculture species is a limited, unsustainable resource with volatile pricing. To determine if alfalfa could be a source of protein for use in aquaculture, ARS scientists in Saint Paul, MN, and collaborators from the University of Minnesota pressed fresh alfalfa foliage to produce a juice and then concentrated the proteins by various methods. A heat treatment resulted in a protein concentrate with the most favorable amino acid profile that did not contain antinutritional factors commonly found in proteins from other plant sources like seed meals. Feeding studies with yellow perch and rainbow trout found that both species accepted alfalfa protein concentrate in feeds. Approximately 800 pounds of protein concentrate can be produced from an acre of alfalfa, and the remaining plant residue can be used as a feed ingredient for cattle, or as a bioenergy feed stock. This research on alfalfa protein concentrate provides the aquaculture industry with the promising development of a sustainable alternative to fishmeal in diets and the alfalfa production industry with a new, high-value use for their crops. (NP215, C4, PS4C, Project No. 5062-12210-003-00D)

AlkarXL, a new tall wheatgrass cultivar for use on saline, semiarid lands. With urban spread in the western United States, farmers and ranchers rely increasingly on marginally productive soils that are frequently high in salt to produce forage. Tall wheatgrass is one of the most salt tolerant grasses used for fall and winter livestock grazing on dryland salty soils, but the yield and quality of this forage for

livestock production needed improvement. ARS scientists in Logan, UT, in collaboration with seed companies, developed and released 'AlkarXL', a new tall wheatgrass cultivar with better forage quality for use on saline soils. Across multiple locations, years, and harvests, AlkarXL produced 3.02 tons per acre of forage, which was on average 14 percent greater than 5 other common tall wheatgrass cultivars. AlkarXL's protein content of 6.2 percent after harvest in the summer was 11 percent greater than these other cultivars. AlkarXL also had higher protein in the fall after regrowth (12.8 percent) than these cultivars and was well above the 7 percent protein level needed by grazing ruminant livestock. Seed of AlkarXL is now commercially available, providing an improved forage grass for marginal salty rangelands. (NP215, C2, PS2A, Project No. 2080-21000-018-00D)

Grass-Cast: A new decision-support tool helps livestock producers improve productivity from rangelands. Western U.S. livestock producers on rangelands can best manage their operations when they use flexible stocking strategies to match the forage needs of their animals with the forage availability on their land. Because forage growth varies greatly depending on precipitation, producers need ways to predict how much forage will be available to take advantage of flexible stocking strategies. ARS scientists from Cheyenne, WY, and Fort Collins, CO, in collaboration with Colorado State University, the University of Arizona, and the National Drought Mitigation Center, developed Grass-Cast, a grassland productivity forecasting tool. Grass-Cast uses year-to-date climate data and seasonal precipitation outlooks to forecast forage production for rangelands at the 6 x 6-mile spatial resolution. Grass-Cast was publicly released in 2018 for the Northern Plains, in 2019 for the entire Great Plains, and in 2020 for New Mexico and Arizona. The public release included the co-development of a new website (https://grasscast.unl.edu), which was recently expanded in 2020 and is co-managed by the USDA Northern Plains Climate Hub and the National Drought Mitigation Center at the University of Nebraska. Grass-Cast has garnered widespread media coverage at regional and national levels, including

a USDA official press release, blog posts, radio news stories, and stories in agricultural newspapers and websites. Livestock producers are interested in having Grass-Cast expanded to the Great Basin, California, and even Canadian prairies. (NP215, C3, PS3B, Project No. 3012-21610-003-00D)

Early alfalfa establishment in silage corn can increase dairy farm profitability. Corn and alfalfa are major crops grown in rotation on dairy farms in the United States. The same alfalfa crop lasts several years, but the conventional practice of planting alfalfa in the spring after a corn crop results in lower yields and farm profitability the first year because the alfalfa is not fully established. ARS and University of Wisconsin scientists in Madison, WI, developed an alternative system where alfalfa is established a year earlier by interseeding into corn. This results in full alfalfa production the following year. The scientists recently used crop yield data, estimated feed values, and farm production costs to show the benefits of the new interseeding alfalfa production system. It can increase net returns by about 15 percent compared to the conventional spring-seeded alfalfa system. The early alfalfa establishment also improves forage yields and provides cover crop benefits for protecting soil and water resources. Dairy farmers will be able to use this new crop production approach to increase their economic and environmental sustainability. (NP215, C4, PS4C, Project No. 5090-12210-001-00D)

Managing Midwest dairy forage production systems to maintain soil carbon. The U.S. dairy industry is committed to environmental stewardship goals, including neutral or reduced carbon emission from dairy farms. Research is needed to determine the opportunities on farms to reduce carbon emissions and sequester carbon, including soil carbon storage. ARS researchers in Saint Paul, MN, monitored carbon balances for 9 years on three fields on a large dairy that uses both solid-liquid separation and anaerobic digestion for manure management. Under the current alfalfa-corn silage production with conventional tillage and inputs of liquid dairy manure, the field soils were losing

carbon, with losses more than three times greater under corn silage than alfalfa. To maintain a neutral carbon balance in corn silage, the equivalent of 70 percent of the carbon removed in harvested corn would have to be returned to fields. That value is 30 percent for alfalfa. However, returned carbon should be even greater to have net soil carbon storage. This rate of carbon returns to fields may require changes in tillage, crop rotation, and manure management, all of which may be a challenge given how such changes affect other enterprises on the farm. These results are being used by scientists developing decision-aid tools that can evaluate whole-farm carbon uses and balances, by dairy producers to understand the tradeoffs in their carbon management, and by dairy industry stakeholders supporting carbon sustainability goals. (NP215, C1, PS1A, Project No. 5062-12210-003-00D)

Development of genomic tools to identify gastrointestinal parasite resistant sheep. Perhaps the most important means of parasite control is an animal's natural resistance. Genetic resistance to parasitic nematodes varies among individual animals within a breed and is moderately heritable. The ability to identify genetic markers of animal resistance to parasites will have wide benefit in the sheep industry as parasites become increasingly resistant to dewormer treatments. A team led by ARS researchers in Booneville, AR, used identified gene DNA sequence variation that was associated with resistance to gastrointestinal parasites in sheep. The team is funded by NIFA's Organic Agriculture Research and Extension Initiative and included colleagues from Louisiana State University, Virginia Tech, Katahdin Hair Sheep International, University of Nebraska-Lincoln, and University of Idaho. This information is important to producers, scientists, veterinarians, and extension specialists to increase natural parasite resistance in sheep and to reduce the use of dewormers, which can in turn reduce parasite resistance to dewormers and extend their effectiveness. (NP215, C4, PS4B, Project No. 6020-21310-011-00D)

Mixing alfalfa and birdsfoot trefoil into tall fescue pastures can improve growth performance and economic return of beef cattle. High nitrogen fertilizer costs and increased emphasis on environmental stewardship have renewed interest among beef producers in the western United States in integrating legumes into their high-productivity, irrigated pastures. However, these producers need to know how forage and livestock growth on grass-legume mixtures compare to that on conventional grass-only pastures fertilized with nitrogen. ARS researchers in Logan, UT, measured herbage mass, nutritive value, steer growth performance, and economics of tall fescue pastures mixed with alfalfa or birdsfoot trefoil compared to fescue-only pastures with nitrogen fertilizer. The grass-legume mixed pastures had slightly less herbage, but nutrition and steer growth performance were better than with fertilized grass pastures. Without the added cost of fertilizer, economic returns for the grass-legume pastures were 2.4 (trefoil) and 1.7 (alfalfa) times greater than the fertilized grass pastures and were competitive with many other crops grown in the region. These novel grass-legume mixtures can help U.S. beef farmers increase the forage and livestock productivity of their pastures and rangelands while reducing dependence on petroleum-based commercial fertilizer. (NP215, C4, PS4B, Project No. 2080-21000-018-00D)

Genes associated with *Verticillium* wilt resistance in alfalfa are identified. *Verticillium* wilt is an alfalfa disease that reduces forage yields by up to 50 percent. An ARS scientist in Prosser, WA, identified DNA sequence changes in two different alfalfa genes that were reliably associated with resistance to *Verticillium* wilt and accounted for one-third of the variation in resistance to the disease. Based on these results, a rapid polymerase chain reaction (PCR) test was developed that may help identify alfalfa plants with increased resistance based on genetic differences. The PCR assay is being further validated in commercial populations. If successful, this new test will accelerate the development of improved alfalfa varieties by making it easier for breeders to select for substantial changes in resistance to *Verticillium* wilt. (NP215, C2, PS2A, Project No. 2090-21000-036-00D)

Determining plant properties and management practices that improve rangeland restoration after wildfire. Grasses are critical plants used in seeding rangeland in the western United States for restoration after wildfire and where invasive species threaten ecosystem function. These rangelands vary widely through the year in soil temperature and moisture conditions that affect grass seedling establishment, and grasses vary inherently in how well they establish. ARS researchers in Boise, ID; Burns, OR; Fort Collins, CO; and Woodward, OK; along with collaborators at the University of California, found that seeds planted later in the fall have a much higher chance of surviving winter mortality, and that diversifying the seed mix is key to ensuring that at least some seedlings successfully establish. ARS researchers in Logan, UT, examined the effect of soil water on root and shoot growth of seven grass species. Three species increased both root and shoot biomass in response to water, while four other grasses increased shoot growth but not root growth. They also found that higher seed mass produced seedlings with more shoot and root biomass that favors short-term growth, while less seed mass produced seedlings with greater surface area of leaves and roots that is better for long-term growth. These findings can improve successful seedling establishment by public land management agencies, help develop more recalcitrant native grass species, and match grass populations to specific site conditions—all to improve re-seeding efforts and make western rangelands more fire and weed resistant. (NP215, C1, PS1A, Project No. 2080-21000-018-00D and No. 2052-13610-014-00D)

Napier grass (elephant grass) as a bioenergy feedstock. Napier grass has the highest biomass productivity of any grass for cropping in the southeastern United States, but management practices to optimize its use in the production of bioethanol are needed. Over a 5-year period, ARS researchers in Tifton, GA, and Peoria, IL, compared how harvest management affected bioethanol production. If the crop was fertilized in May and harvested once per year in December, production was consistent over 5

years. The ethanol yield per acre using one harvest per year was 962 gallons in the second growing season and 1,368 gallons in the fourth season, which out-yields the national average yield of 180 bu/525 gallons from corn produced for ethanol. In contrast, two harvests per year (June and December) led to dramatic declines in production beginning in Year 3. This work will help bioethanol producers and farmers in the Southeast maximize Napier grass as a competitive bioethanol feedstock. (NP215, C4, PS4C, Project No. 6048-21000-030-00D)

## Use of noninvasive sampling procedures can help diagnose livestock exposure to poisonous plants.

Larkspurs, lupines, and death camas can be acutely toxic to livestock and are serious poisonous plant problems in western North America. Poisoning of livestock by plants often goes undiagnosed because there is a lack of appropriate or available specimens for analysis. ARS researchers in Logan, UT, developed procedures to detect toxic compounds in easily obtainable animal samples (rumen contents, ocular fluid, earwax, hair, oral fluid, and nasal mucus) to help easily diagnose cattle that consumed toxic plants. The advantage of using theses samples is that collecting them is noninvasive and relatively easy—no special equipment is required, and untrained personnel can easily collect the samples. These methods will be valuable for livestock producers, extension agents, veterinarians, and especially laboratories to help diagnose animals that are poisoned by common toxic plants found in rangeland environments. (NP215, C4, PS4D, Project No. 2080-32630-014-00D)

**Survey of pollinating insects in centipedegrass lawns.** In the United States, turfgrasses are a major component of the landscape and cover more than 40 million acres. Centipedegrass is a warm-season turfgrass that is often grown in the southeastern United States. Recently, honeybees were documented collecting pollen from the flower structures of centipedegrass. To understand the role of turf species such as centipedegrass in supporting pollinators, ARS researchers in Tifton, GA, and scientists from the

University of Georgia surveyed bees in centipedegrass lawns in central and southern Georgia. Numerous bees were observed, of which 79 percent were Lasioglossum species (sweat bees). Minor species observed included long-horned bees; other species of sweat bees, including metallic green sweat bees; bumble bees; leafcutter bees; and small carpenter bees. These data indicate that diverse bees are residing in or near lawns and forage in and around the lawns seeking centipedegrass flowers. The knowledge that many bees are present in centipedegrass lawns indicates that homeowners and landscape managers should apply insecticides conservatively, since certain insecticides are toxic to foraging bees in lawns. (NP215, C1, PS1A, Project No.6048-21000-030-00D)

# Evidence of consistent and sustained profitability in precision agriculture cropping systems.

Targeting management practices and inputs with precision agriculture has high potential to meet some of the grand challenges of sustainability in the coming century. The benefits include improving crop profitability and reducing environmental impacts, but its reputation for high cost limits its popularity. To better understand long-term effects of precision agriculture on crop profitability, ARS scientists in the Columbia, MO, Long-Term Agroecosystem Research (LTAR) network site monitored a 90-acre field in central Missouri for over a decade under conventional management (1993–2003) and then for another decade under a precision agriculture system (2004–2014). Conventional management was a cornsoybean rotation, annual tillage, and uniform fertilizer and herbicide inputs. Key aspects of the precision system were no-tillage, cover crops, winter wheat instead of corn on areas with shallow topsoil and low corn profitability, and variable-rate fertilizer (nitrogen, phosphorus, potassium, and lime) applications. Results indicated that precision agriculture sustained profits in 97 percent of the field without subsidies for cover crops or payments for enhanced environmental protection. In a separate study, ARS scientists in Pendleton, OR, applied a specialized mathematical model to multiple years of yield map data from a dryland field in South Dakota (corn-soybean rotation) and an irrigated field in Georgia (corn, soybean,

and peanut). In both cropping systems, this analysis method effectively revealed patterns of productive and unproductive parts of the field. This information can be used to target more efficient crop management techniques, and these results help growers gain confidence in the economic success of precision agriculture management and conservation practices. (NP 216, C1, PS1D, Project No. 5070-12610-005-00D and Project No. 2074-12210-001-00D.)

Long-term data show crop diversity improves corn yields and enhances drought resilience. ARS researchers in Akron, CO; Lincoln, NE; Brookings, SD; and Beltsville, MD, in collaboration with scientists from universities across North America, compiled 347 seasons of data from 11 long-term experiments that spanned the continental precipitation gradient. Researchers found that more diverse crop rotations increase corn grain yield across all growing conditions by an average of 28 percent and that during drought years, corn yield losses were reduced from 14 to 90 percent. Crop rotation diversification is a risk-reduction strategy for producers under increasingly stressful weather conditions. (NP216, C1, PS1A, Project No. 3010-12210-004-00D, Project No. 3042-11210-003-00D, Project No. 8042-21660-005-00D and Project No. 3080-12620-005-00D

A framework for redistributing manure to meet regional production, economic, and environmental goals. Managing manure is one of the most difficult challenges of modern agriculture because excess manure can negatively affect water quality for human and environmental health. ARS scientists in Las Cruces, NM, and Tifton, GA, led a collaboration across 10 sites in the ARS Long-Term Agroecosystem Research (LTAR) network to classify 3,109 counties as "sources" of manure nitrogen and phosphorus from confined livestock production, or "sinks" that can use these nutrients to improve crop or hay production. The resulting four "manure sheds" represent various regional combinations of beef, dairy, poultry, and swine industries, and differ in the transport distances needed to productively use

manure, from an average of 90 miles for a cattle-dominated system to 200 miles for a poultry-dominated system. Diverse stakeholders, including crop and livestock farmers, university extension staff, developers of manure-treatment technologies, and policymakers, can use the manure shed concept to develop strategies for recycling manure and transforming it from a liability to a valuable resource. (NP216, C1, PS1C, PS1D, C3, PS3A, Project No. 3050-11210-009-00D and Project No. 6048-11130-005-00D

An inexpensive portable high throughput phenotyping (HTP) system for rapid crop assessment and precision management. Phenotyping is the process of collecting data about an organism's physical characteristics, which is useful for research in breeding better crop species or assessing plant response to stresses like drought and heat. Manually collecting this data is very time-consuming and requires a lot of labor, so scientists are working on ways to increase throughput by automating these measurements. An ARS researcher from Maricopa, AZ, developed and tested a low-cost wireless HTP system powered by a solar rechargeable battery. This system measures vegetation index, canopy temperature, and height from a multispectral camera, an infrared (IR) thermometer, and mini-LiDAR sensors, respectively. This portable system can be mounted to unmanned aerial or terrestrial vehicles (drones) for use in fields or to indoor platforms for use in greenhouses or vertical farms. Because it is wireless, the data can be easily monitored from a smartphone or computer. The HTP system enabled automated irrigation control in a plant growth chamber based on soil water condition and automated collection of phenotypic data for months. This innovative data collection system has the potential to be adapted for use in commercial precision agriculture. (NP216, C1, PS1A, Project No. 2020-11000-013-00D)

**Perennial crops improve crop, livestock, and timber production.** ARS researchers in Mandan, ND, teamed up with scientists in 9 countries to show that: 1) a change from annual to perennial crops over 20

years led to an average 20 percent increase in carbon in the top 12 inches of soil and a 10 percent increase in the top 40 inches; and 2) woody crops were most effective at increasing soil carbon. ARS researchers in Ames, IA, along with university collaborators, showed that widely-spaced oak and pecan trees near Fayetteville, AR, removed and stored in their above-ground biomass 0.75 and 0.2 Mg carbon per hectare per year, respectively, while enabling simultaneous livestock and timber production. This information is useful to producers who wish to combine agricultural production with environmental benefits of carbon removal from the atmosphere, and to people who are developing carbon offset markets that enable farmers to receive additional payments for this environmental service. (NP216, C1, PS1C, C3, PS3B, Project No. 3064-21660-004-00D and 5030-11610-005-00D)

Making Southwestern agricultural systems more resilient to weather variations. Weather-related challenges in southwestern U.S. communities and ecosystems include crop loss, extreme drought, variability in rangeland production, and wildfire. As members of the USDA Southwest Climate Hub (SW Climate Hub), ARS scientists in Las Cruces, NM, developed an online dust mitigation handbook with the Natural Resources Conservation Service (NRCS) and contributed to the AfterFire toolkit, an online post-fire resource for water managers. They also hosted an urban tree adaption workshop, expanded Grass-Cast (a forage production forecasting tool) to New Mexico and Arizona, conducted a survey of cattle producers to understand vulnerabilities to climate change and adaptation options, and co-launched the Drought Learning Network with climate scientists and land managers. These activities will assist farmers, ranchers, foresters, and other land managers in developing and implementing strategies to adapt to the impacts of extreme weather. (NP216, C2, PS2C, Project No. 3050-11210-009-00D)

Pelletizing makes manure easier to transport from livestock facilities to fields. ARS researchers in Mississippi State, MS, evaluated the effects of using pelleted manure as a source of fertilizer nutrients and organic matter in corn, soybean, and cotton fields over 4 years. Grain and lint yields were comparable between pelleted manure and inorganic fertilizer nitrogen at equivalent nitrogen rates, while pelleted manure reduced nitrate percolation below the root zone. The practice of applying pelleted manure reduces the need for inorganic fertilizers and enables growers to maximize the return on their nutrient management practices while minimizing adverse impact on water quality. (NP216, C1, PS1C, C3, PS3B, Project No. 6064-21660-001-00D)

Mapping wheat grain protein concentration on-combine. Protein concentrations affect wheat quality and price, but the ability to map concentrations during harvest is unrealistic based on the high cost (more than \$20,000) of commercially available spectrometers. ARS scientists in Pendleton, OR, adapted a moderately-priced reflectance spectrometer (less than \$5,500) for use on a combine to measure and map the protein concentration of wheat during harvest. When calibrated, this instrument produced a protein map that was comparable to a map derived from a more expensive instrument. Having a less costly instrument for mapping protein across fields will enable more farmers to implement precision nitrogen management and to segregate and blend grain to achieve the desired quality. (NP216 C2, PS2B, Project No. 2074-12210-001-00D)

Biosensor enables farmers to more confidently use seed meal as a soil amendment. Seed meal produced from canola and mustard can reduce pathogen pressure and suppress the germination of weeds by releasing glucosinolates (GCSs) and isothiocyantes (ITCs) into the soil. However, the widespread use of canola and mustard-based seed meal is hampered because these compounds can also suppress the germination of crop plants. Scientists in Corvallis, OR, developed a bacterial biosensor that detects

biologically relevant concentrations of GCSs and ITCs in soil at much lower cost compared with advanced instrument methods. This technology is being developed into a method that can rapidly screen soils for the presence of ITCs, allowing growers to determine when it is safe to replant fields after the application of seed meals without suppressing crop germination. (NP216, C2, PS2B, Project No. 2072-12620-001-00D)

Managing brush encroachment in desert grasslands. Arid grassland is degraded when woody plants crowd out grasses, resulting in intensified erosion. ARS scientists in Las Cruces, NM, established a collaborative long-term distributed experiment to measure the conservation effects of brush management in 45 treatment areas across southern New Mexico. Analysis of data from 5 to 10 years of vegetation monitoring indicates that grass restoration is favored at higher elevations and on specific ecological sites, whereas shrub reinvasion after treatment is more likely in soils with higher clay content and when shrub cover was initially high. This study will help land managers improve the success rate of grassland restoration efforts by predicting the effects of brush management on conservation and forage production outcomes. (NP216, C1, PS1B, Project No. 3050-11210-009-00D)

Winter flooding of rice fields improves soil biology and waterfowl habitat but increases carbon footprint. ARS researchers in Jonesboro, AR, and Mississippi State, MS, evaluated trade-offs associated with a flooded winter fallow management option that is recommended by some conservation planners to provide waterfowl habitat. Winter flooding is beneficial for soil health as measured by soil biological activity, microbial groups, and plant nutrients, apparently due to fecal deposition by waterfowl as they frequent the flooded fields. However, methane gas emissions are higher during flooded than non-flooded fallow and can equal as much as 20 to 30 percent of those produced during the rice growing season. ARS researchers also found that methane can be reduced by up to half during the

growing season, relative to conventional practices, when rice farmers use intermittent flooding, a water-conserving irrigation practice. These results show that methane emissions created by winter flooding can be offset by careful irrigation management during the rice growing season while protecting grain yield and groundwater resources. This information provides guidance to rice farmers who wish to manage their cropping system for profitability and environmental sustainability. (NP211, C, PS1C, NP216, C1, PS1A, C3, PS3B, Project No. 6064-21660-001-00D and Project No. 6024-13000-003-00D)

Cover crops and compost increase soil health in long-term vegetable rotations. Soil organic matter often declines in tillage-intensive vegetable production systems, which can reduce soil health and productivity. Farmers working to improve soil management strategies need long-term information on how organic matter inputs from compost and cover crops affect soil organic matter levels. ARS researchers in Salinas, CA, and Beltsville, MD, evaluated changes over 8 years in soil organic matter in several vegetable rotations in the Salinas Valley that differed in the amount of organic matter input from winter cover crops and yard-waste compost. Although compost and cover crops both increased soil organic matter levels, frequent cover cropping had a greater impact on the type of organic matter that is more closely linked to increased crop yields and nutrient cycling. This information is useful to vegetable producers who seek to improve the profitability of their cropping systems through improved soil health. (NP216, C2, PS2A, PS2B, Project No. 2038-21620-014-00D and Project No. 8042-21660-005-00D

High-quality forage can be produced from cover crops in place of summer fallow. Summer fallow degrades soil quality and is a non-sustainable cropping practice. Planting cover crops in place of fallow helps improve soil quality and can also provide a source of high-quality forage. ARS researchers in Sidney, MT, planted a 10-species crop mix (buckwheat, cowpea, flax, lentil, millet, mustard, pea, radish, sorghum, and turnip) in place of fallow in 2-year durum wheat rotations from 2014 to 2019. When

harvested in early summer, this mixture produced prime quality forage at an average of 1.5 tons per acre. After forage harvest, regrowth of cover crops terminated by killing frost averaged 2.9 tons per acre of unharvested standing cover. Given the growing interest among producers in incorporating a diverse cover crop mix into their dryland cropping system to improve soil biological function, these results provide information about how a cover crop might also provide an immediate economic return when used as a forage. (NP216, C1, PS1A, C2, PS2A, C3, PS3B, Project No. 3032-13210-007-00D)

Reducing our carbon footprint with renewable fuels grown on marginal land. ARS researchers in University Park, PA, showed that ethanol could be produced from barley with a carbon footprint less than half that of gasoline, allowing it to meet the advanced fuel standard of the U.S. Environmental Protection Agency. ARS scientists in Mandan, ND, along with scientists at Michigan Tech and the U.S. Department of Transportation, demonstrated that growing oilseeds in place of fallow in non-irrigated areas of the Great Plains reduces greenhouse gas (GHG) emissions, increases soil carbon, and could boost regional farmer incomes from \$127 million to \$152 million per year through jet fuel production. ARS scientists in Lincoln, NE, and Fort Collins, CO, determined that, compared to GHG-neutral continuous corn under conservation management, long-term (16 years) switchgrass systems mitigate GHG emissions during feedstock production by capturing more carbon in soil and mitigating nitrous oxide loss. ARS researchers in Temple, TX, identified genetic information in switchgrass that enables it to adapt to different growing conditions across a regional gradient from Texas to South Dakota. This research provides farmers with information about how to diversify income through emerging renewable fuel markets and provides policy makers with data needed to create programs to support renewable fuel production. (NP216, C1, PS1A, C3, PS3C, Project No.8070-66000-001-00D, Project No. 3064-21660-004-00D, Project No. 3042-11210-003-00D, Project No. 3098-11000-001-00D, Project No. 3012-11120-001-00D)

Improved methods to provide fertilizer recommendations and predict the effects of soil conservation practices on soil erosion. ARS scientists in Columbia, MO, along with scientists at 8 Midwest universities, evaluated 31 publicly available tools used in 49 field experiments to determine nitrogen fertilizer recommendations for corn. They found that yield-goal based tools recommended more nitrogen than necessary and that the tools using soil nitrate tests or measurements of leaf color came closest to the economically optimal nitrogen rate. ARS researchers in Tifton, GA, demonstrated the importance of using variable-rate simulated rainfall tests, rather than the more common constant-rate simulation to measure soil erosion and nutrient loss under different tillage practices. Typical rainfall events were reflected more accurately when rainfall rate was varied during the simulation, while the advantage of no-till practice compared with conventional tillage was found to be diminished during high-intensity rainfall. This analysis of scientific methods is useful to scientists who want to improve the accuracy of their models as well as to land managers who want to improve their nitrogen management and soil conservation practices. NP216, C1, PS1A, C2, PS2C, C3, PS3A, Project No. 5070-12610-005-00D and Project No. 6048-11130-005-00D

## Evidence grows that reducing tillage intensity and planting cover crops improves soil health.

Improving soil health builds the capacity of the soil to function as a vital, living ecosystem that sustains plants and animals, but it is a challenge to adequately measure the improvement that management practices have on soil health. ARS scientists in Ames, IA, and Columbia, MO, conducted a meta-analysis of 302 published studies throughout the United States to assess the effects of chisel plowing (CP), no-tillage (NT), and perennial cropping systems (PER) relative to moldboard plow (MP) on seven soil health indicators: soil organic carbon, microbial biomass carbon, microbial biomass nitrogen, soil respiration, active carbon, beta-glucosidase activity, and soil protein within 4 soil depth increments.

Overall, reducing tillage by converting from MP to CP improved topsoil organic carbon, microbial biomass carbon, and respiration, whereas converting from MP to NT significantly increased all seven soil health indicators in the topsoil. Below the topsoil, NT resulted in greater microbial biomass, microbial carbon, respiration and beta-glucosidase activity relative to MP. Based on this analysis, reducing tillage intensity, planting cover crops, and/or minimizing crop residue removal within annual cropping systems can significantly improve soil biological health in the United States. Soil biological indicators are sensitive to management practices, confirming their utility in soil health assessment. Scientists can use this analysis to choose soil health measurements in their experiments that test the sustainability of different agricultural practices, and farmers will have added confidence that recommended practices improve soil health in a measurable way. (NP216, C1, PS1A, Project No. 5030-12210-003-00D)

Powerful tools and techniques for monitoring rangeland production systems improve management and lower production costs. Standardized approaches for monitoring rangelands are needed to allow land managers and public land agencies to collect and share data that address numerous rangeland management and policy needs. ARS scientists in Las Cruces, NM, led the expansion of the rangeland monitoring program that directly supports the Bureau of Land Management (BLM) and Natural Resources Conservation Service (NRCS) national inventory and monitoring programs and the interagency National Wind Erosion Research Network. The Monitoring Manual for Grassland, Shrubland, and Savanna Ecosystems was published online, and physical copies were distributed to field staff. Statistical analysis tools and datasets were used by BLM and NRCS to produce reports and make management decisions regarding wildlife habitat suitability, evaluate conservation practice effectiveness, and improve grazing management systems across the continent's rangelands. (NP216, C1, PS1A, PS1B, Project No. 3050-11210-009-00D)

Removal of corn residue in semiarid irrigated systems reduces water use efficiency. Removing residue from corn fields for biofuel production offers an opportunity to increase farmer profits, but there are potential tradeoffs for water availability and crop performance. A multi-year collaboration between ARS researchers in Akron, CO, and scientists from Colorado State University compared the effects of two tillage practices (no-till and conventional), and two corn residue management practices (harvested and retention) on grain yields, water infiltration, evaporation, and soil quality. Corn grain yields increased and evaporative water losses were reduced with residue retention, especially under no-till. Water infiltration into the soil was higher with residue retention, resulting in higher water content in the soil at planting. The study suggests that high rates of crop residue removal under limited irrigation can negatively affect water conservation and yields, and that tradeoffs surrounding residue export need to be considered. These results will help irrigated corn growers in the region make better residue management decisions. (NP216, C1, PS1A, Project No. 3010-12210-004-00D)



## **CROP PRODUCTION AND PROTECTION**

#### **National Programs:**

- Plant Genetic Resources, Genomics and Genetic Improvement, NP 301
- Plant Diseases, NP 303
- Crop Protection and Quarantine, NP 304
- Crop Production, NP 305

Redesigning soybean meal for nonruminant animals. Soybean is the top global source of protein meal for animal feed. Soybean seeds, however, contain carbohydrate compounds called raffinose family oligosaccharides (RFOs) that prevent effective digestion of soybean meal in nonruminant animals. To combat this problem, ARS scientists in West Lafayette, IN, and Columbia, MO, collaborated with university partners to identify and incorporate new genes to reduce or eliminate RFOs in soybean meal. A soybean population treated with an agent to induce mutations was screened to generate desirable genes in the RFO biosynthesis pathway. A new mutant was recovered from this screen that reduced RFOs from 5 to 6 percent of total carbohydrates to below detectable levels when used in combination with other modified RFO soybean genes. Essentially, a "No-RFO" soybean was created. Field studies established the stability of the genes, and feeding trials demonstrated that "No-RFO" soybean meal improved poultry growth and nutrition. The researchers discovered close associations of the genes

controlling the modified RFO plant type with molecular markers that can be used to detect the variant alleles during breeding. These discoveries have been shared with public soybean breeders, as have the modified seeds and marker information that can be used to enable marker-assisted selection for improved soybean meal that can add value to soybean by improving the metabolizable energy of the meal. (NP301, C1, PS1B, Project No. 5020-21000-008-00D and 5070-21000-040-00D)

A covert killer of macadamia trees discovered in Hawaii. Currently more than 17,000 acres (approximately 1.2 million trees) of macadamia nut are grown in Hawaii, constituting a \$42 million/year industry. Early detection and identification of newly emerging diseases are the critical first steps for protecting this valuable crop. ARS researchers in Hilo, HI, published the first report of *Phytophthora heveae*, a plant pathogen, as the cause of macadamia quick decline and the first report of this pathogen on any host plant in Hawaii. *Phytophthora heveae* has a wide host range, and its presence in Hawaii also poses a potential risk for avocado, mango, and cacao. ARS researchers are working with macadamia growers and researchers to assess the impact of Phytophthora diseases and develop improved management practices that minimize tree loss in mature orchards and maintain the economic viability of the macadamia industry. Commercial macadamia production originated in Hawaii, which now produces nuts that are considered the global "gold standard" for quality and flavor. (NP301, AP 2018-2022, C2, PS2a, Project No. 2040-21000-016-00D)

Characterization of the changes responsible for increased stalk pathogen- and drought-resistance in brown midrib 12 (bmr12) sorghum. ARS researchers in Lincoln, NE, examined how two brown midrib lines (bmr6 and bmr12) and the corresponding normal sorghum line responded to two stalk diseases (Fusarium stalk rot and charcoal rot) under drought stress and adequate water conditions.

Bmr12 plants have impaired lignin synthesis; lignin is a cell wall component that plays a role in plant

defenses against drought and pathogen attack. Bmr12 plants had fewer disease symptoms under drought conditions than normal plants or even bmr12 plants under adequate water conditions. Further analyses show that bmr12 had increased defense signals under drought conditions, which suggested these plants were already prepared for a pathogen attack. This research showed that bmr12 sorghum with reduced lignin may have improved ability to serve as a forage and bioenergy crop. The identification of genes and pathways in bmr12 plants that direct lignin synthesis may lead to the development of more climate-and disease-resilient sorghum hybrids. (NP301, C3, PS3A; NP303, C3, PS3A, Project No. 3042-21220-033-00D)

## Identification of romaine lettuces with reduced browning discoloration for fresh-cut processing.

Lettuce is one of the most valuable fresh vegetables and one of the top 10 most valuable crops in the United States, with an annual farm-gate value of more than \$2.5 billion. Fresh-cut lettuce is the primary ingredient of the increasingly popular packaged, ready-to-eat salads; however, discoloration (browning) represents a major challenge that limits its quality and shelf life. Processors who lack effective browning control methods are relying on modified atmosphere packaging (MAP) to achieve low oxygen atmospheric conditions and maintain the shelf life. ARS researchers in Salinas, CA, and Beltsville, MD, identified lettuces with limited browning that will be used in breeding programs and to help identify genes associated with limited browning. These findings are of great benefit to the U.S vegetable industry. (NP301, C1, PS1A, PS1B, Project No. 2038-21530-002-00D)

## Under-the-radar dengue virus infections in natural populations of Aedes aegypti mosquitoes.

Metagenomics has helped identify dengue virus in Florida prior to any human infection. ARS researchers in Stoneville, MS, have demonstrated the ability to monitor vector-borne diseases ahead of outbreaks using metagenomics. To date, the current U.S. public health system's response to outbreaks

has been largely reactive, but this research shows that by monitoring mosquito populations, it may be possible to identify emerging mosquito-borne diseases in high-risk, high-tourism areas of the United States to enable proactive, targeted vector control before potential outbreaks occur. (NP301, C3, PS3A, Project No. 6066-21310-005-00D)

New hybrid hemlock 'Traveler' is resistant to insect pests. The native hemlock, *Tsuga canadensis*, plays an essential role in forest ecosystems and cultivated landscapes; however, it is susceptible to feeding damage by the hemlock woolly adelgid, which has caused widespread loss of hemlocks in wild and cultivated settings. ARS scientists at the U.S. National Arboretum in Washington, D.C., developed and introduced the first interspecific hybrid hemlock to the trade. 'Traveler' is a cross between the Chinese hemlock and the Carolina hemlock. It was explicitly bred for resistance to hemlock woolly adelgid and selected for its regular and slightly pendulous shape and its moderately slow growth rate. It is currently being propagated under license at cooperator nurseries and promises to be a valuable addition to residential, commercial, and forest landscapes. (NP301, C1, PS1B, Project No. 8020-21000-071-00D)

## Genetic mapping of traits associated with pollinator visitation, yield, and seed size in sunflowers.

Pollination by wild bees increases yields of both oilseed and confection sunflowers, and bees prefer sunflowers with shorter florets for easier nectar access. In addition, seed size (length and width) is important in the confection sunflower market. ARS scientists in Fargo, ND, and University of Colorado-Boulder colleagues mapped genes associated with both floret depth and seed size to specific chromosomal locations in cultivated sunflowers and designed genetic markers to use in identifying desirable lines carrying both those traits. Floret length and seed length are correlated, but when the team mapped these traits, they discovered that the genetic bases for the two traits are largely independent and

can be improved separately. Markers developed for seed and floret length will improve private- and public-sector breeding by allowing effective selection of lines that are attractive to bees and possess desirable seed size and shape for the confection sunflower market. These cultivars will provide ecosystem services for bees and economic value for sunflower producers and consumers. (NP301, C1, PS1B, Project No. 3060-21000-043-00D)

Understanding the genome sequence of pecan. Understanding the detailed genetic makeup of pecan is critical to advancing breeding efforts. The whole genomes of the pecan cultivars 87MX3-2.11, 'Lakota', 'Elliott', and 'Pawnee' were sequenced by ARS researchers in College Station, TX. This also includes sequencing the pecan chloroplast, a small, extra-nuclear genome that is critical for photosynthesis and that is generally passed from the mother to the offspring. Because of this unique inheritance pattern, chloroplast genomes can be used to trace maternal origin over many generations. Sequence data provide the genetic blueprint of gene location and function, thus establishing foundational information to support pecan genetic research for years to come. This accomplishment is critical for future work to establish the origin of pecan in North America, to define evolution of the species, and to guide ongoing efforts to improve pecan trees through breeding. (NP301, C4, PS4A, Project No. 3091-21000-039-00D)

Evaluation of blueberry genetic resources identifies fruit fly resistance. Spotted wing drosophila fruit fly appeared in the United States in 2009 and now causes more than \$511 million in damage annually to fruit production in Western states. ARS scientists from Corvallis, OR, and Poplarville, MS, searched blueberry genetic resources for resistance to that fruit fly. Twenty-nine blueberry species were tested with a bioassay for resistance to feeding by the fruit fly larvae and adults. Ten blueberry species were resistant to feeding by the fruit fly; three of those species are indigenous to East Asia, in the fruit fly's native range. Blueberry species from Central and South America were also resistant. Most

highbush blueberry cultivars were susceptible, but rabbiteye and other blueberry cultivars with smaller, firmer fruit types were resistant. These findings will enable blueberry breeders to identify parental lines to cross and produce new blueberry cultivars resistant to this fruit fly. (NP301, C1, PS1a, Project No. 2072-21000-049-00D).

Galena Russet, a new potato variety with high yield and attractive tubers suitable for multiple uses. U.S. potato production is valued at \$4 billion annually. However, the industry needs new potato varieties with high yields and better processing quality. ARS scientists in Aberdeen, ID, along with researchers at experiment stations in Idaho, Oregon, and Washington, released and filed for Plant Variety Protection for 'Galena Russet', a new potato variety that produces high yields in both early and full-season harvests, allowing for use over more growing areas. 'Galena Russet' has excellent processing characteristics for fries due to cold sweetening resistance, allowing for lighter colored fries late in the storage season. This is an improvement over other varieties in which conversion of starch to sugar results in a darker product. 'Galena Russet' has an attractive tuber shape and low levels of defects, making this a good fresh market potato. This new potato variety will greatly benefit the potato industry. (NP301, C1, PS1B, Project No. 2050-21000-035-00D)

Breeding Insight supports ARS specialty crop and animal breeders. ARS specialty crop breeders are often the sole source of publicly available new crop varieties for farmers and growers across the United States and elsewhere. Although specialty crops and animals are a large portion of gross U.S. agricultural revenue, individually these small programs have not had access to innovations that benefited major crop and animal breeding programs and thus have lagged. The ARS Breeding Insight Platform is currently in a pilot phase focused on building support services for six ARS breeding programs (blueberry, table grape, sweet potato, alfalfa, rainbow trout, and North American Atlantic salmon), with the future goal of

expansion to all ARS specialty crops, animal, and natural resource breeding programs. The project identified the key workflows common to these diverse programs and initiated the development of extensive software and genomics to support these efforts. Genomic support was delivered for all programs. A key early success was integrating the leading field data collection tool with the community's leading database. Providing powerful information and genomic tools to ARS specialty crop and animal breeders is helping to improve breeding decisions, meet public demands for more nutritious and flavorful foods, and improve food security for the United States and its trade partners. (NP301, C2, PS2A, Project No. 8062-21000-043-00D)

New food barley variety with a winter growth habit provides additional options for growers.

Winter cereal varieties can provide growers with options to manage soil moisture resources and to minimize crop losses to biotic and abiotic stress pressures. A new winter barley cultivar, 'Upspring', is the first two-row food barley cultivar that will fill this niche. Upspring is the product of breeding by ARS researchers in Aberdeen, ID. The new cultivar is well balanced in yield, winter hardiness, and quality traits with high beta-glucan content. (NP301, C1, PS1B, Project No. 2050-21000-034-00D)

Stopping blue mold fungus decay in apples. Apples are one of the most popular fruits consumed in the United States, and may be stored for up to 12 months. During storage, the blue mold fungus may cause the apple to rot, reducing its quality and marketability. ARS researchers in Beltsville, MD, in collaboration with University of Wisconsin, Pennsylvania State University, and Dartmouth University colleagues, have discovered a way to block the gene in the blue mold fungus that causes apple rot in storage. The apple industry and researchers are using this new knowledge to develop postharvest decay treatments for the blue mold fungus. (NP303, C1, PS1A, Project No. 8042-42430-002-00D)

Combining herbicides with biofumigants for better pest and disease control. The loss of methyl bromide has left growers with few soil fumigant options, particularly for buffer zone areas where conventional fumigants cannot be applied. Alternatives to methyl bromide are urgently needed for both conventional and organic crop production. ARS researchers in Fort Pierce, FL, conducted field trials on tomato and bell pepper plants in Florida using a combination of herbicides and a newly registered preplant biofumigant with the active ingredient allyl isothiocyanate. The treatments resulted in broadspectrum control of pathogens and weeds to a degree that was equivalent to when methyl bromide was used, including lower incidence of bacterial wilt of tomato, lower populations of root-knot nematodes in soil, and fewer nutsedge weeds. Herbicide and biofumigant combinations provide a greatly needed option for controlling crop pests and diseases in both conventional and organic crop production in Florida and elsewhere. (NP303, C3, PS3C, Project No. 6034-22000-043-00D)

Precision thermotherapy lowers leaf spot on strawberry. In small-scale experiments, heat treatment has previously been shown to be an effective way of managing the bacterium that causes angular leaf spot (ALS) on nursery stocks of strawberry. ARS researchers in Fort Pierce, FL, designed, built, and tested a commercial-scale precision thermotherapy unit for applying a new thermotherapeutic protocol that combined two heat treatments, a conditioning thermal treatment and an eradicative thermal treatment. Several trials were conducted in cooperation with commercial nurseries to determine the effects of thermotherapy on plant health and on the natural development of ALS. Overall, precision thermotherapy had a negligible effect on plant growth and yield. When ALS occurred, its effect was always lower in plots that received the thermotherapy treatment. Successfully scaling up thermal treatment of nursery stock to commercial levels could help control pests and diseases while simultaneously reducing or eliminating pesticide use against a broad range of threats to sustainable strawberry production. (NP303, C3, PS3A, Project No. 6034-22000-042-00D)

Integration of linkage blocks of wheat stem rust (Ug99) resistance genes. The finding that genes that support disease resistance in wheat are linked is a great asset for gene stewardship that will serve to extend the life of resistance in modern varieties. ARS researchers in St. Paul, MN, have effectively combined multiple linked genes that are effective against Ug99 and backcrossed these linkage blocks into conventional wheat germplasm. Two resistance genes were combined on one chromosome arm, and two other genes were combined on a different chromosome arm. The two linkage blocks were backcrossed into hard red spring wheat cultivars that previously had been released by the University of Minnesota and South Dakota State University. The Ug99-resistance-enhanced lines have been used to develop new wheat cultivars with multiple Ug99-resistant genes. (NP303, C3, PS3A, PS3B, Project No. 5062-21220-023-00D)

Creation of a genomic database for a major soilborne pathogen. *Rhizoctonia solani* is a soilborne plant pathogen with a wide host range and worldwide distribution. It is difficult to precisely identify the various isolates of the pathogen complex because the pathogen produces no spores. Genomes of 13 isolates from the *R. solani* plant pathogen complex were sequenced, assembled, and annotated, and a genome database was created that is publicly accessible. This database provides scientists with information that is critical for the development of methods for identifying various isolates within the *R. solani* pathogen complex. Identification and differentiation of the many morphologically similar *R. solani* species is necessary to develop appropriate plant disease control measures. (NP303, C3, PS3B, Project No. 8042-21220-181-00D)

**DNA sequence resources for bacterial plant pathogens of economic importance.** The separate pathogens that cause almond leaf scorch, olive quick decline, and citrus greening have significant impacts on crop production worldwide. DNA sequences from these pathogens can be used for detection,

surveillance, and disease tracking purposes. ARS scientists in Parlier, CA, and Byron, GA, with collaborators in Brazil used next-generation sequencing technology to create whole-genome sequencing data generated through multiple short- and long-sequences for pathogen strains associated with olive in California and pecan in Georgia. The scientists were also able to fully sequence a citrus greening pathogen strain from Brazil. This new DNA sequence information is valuable for development of new disease management and diagnostic strategies, and for pathogen detection and quarantine applications. (NP303, C2, PS2A, Project No. 2034-22000-012-00D)

Epidemiological models predict the spread of two severe citrus diseases by hurricanes. Asiatic citrus canker (ACC) and citrus black spot (CBS) cause economic damage and are severe impediments to international trade of citrus as a commodity. ACC, caused by a bacterium, and CBS, caused by a fungus, are both dispersed by rain splash. During a hurricane, such rain splash can be spread over many miles. Two such hurricanes, Harvey in southeast Texas and Irma in southwest Florida, potentially spread ACC and CBS, respectively, in 2017. ARS researchers in Fort Pierce, FL, adapted and extended a previously developed hurricane dispersal model to address both diseases and make predictions for where these infections may have spread due to the hurricanes. The results of these model predictions were presented to regulatory agencies and science advisory committees in Florida and Texas. ARS researchers also developed a model to predict where to look for pathogen entries that were rejected by the hurricane models. As a result, regulatory agencies in both States in collaboration with the USDA Animal and Plant Health Inspection Service have deployed the surveys for early detection of potential spread of these diseases in both States. (2017, NP303, C2, PS2B, Project No. 6034-22000-042-00D)

**First report of grapevine red blotch virus in Idaho.** Some grapevine viruses are detrimental to grapevine health, crop load ratio, fruit characteristics, and ultimately to wine quality, while others cause

only minor issues. ARS scientists in Parma, ID, and University of Idaho collaborators conducted research on grapevine viruses in collaboration with commercial grape growers. This work is the first report on the presence of grapevine red blotch virus (GRBV) in Idaho commercial vineyards. Multiple years of sampling and testing for GRBV indicate the spread of this virus is limited in Idaho. These findings can be used by the grape industry for making vineyard replanting decisions. (NP305, C1, PS1B, Project No. 2072-21000-052-00D)

Plant "organ transplants" offer a new way of delivering genetic engineering solutions to solve crop pest/pathogen problems. There is an urgent need for solutions to control whitefly and the diseases transmitted by it. ARS researchers in Fort Pierce, FL, in collaboration with a private industry partner, developed a method of engineering only a group of plant cells that can be attached to other plants (essentially as a new organ) to produce desired molecules that are secreted into the plant vascular tissue and move throughout the plant. This "new organ" cannot survive away from the plant and does not move from the location where it is attached, thus the harvested commodity (i.e., fruit, nut, etc.) is not genetically engineered. It also cannot form whole plants, seed, or pollen, thus there is no escape of genetic material. The scientists are evaluating the ability of this strategy to cure trees infected with *Huanglongbing* (HLB, aka citrus greening) by engineering similar organs to produce natural peptides and double-stranded RNA that kills the HLB-causing bacterium, and attaching these organs to ornamental and/or horticultural crops. Proof-of-concept has been completed in tomato. This strategy could be adapted as a means to rapidly deliver genetic engineering solutions in an environmentally sustainable and consumer acceptable method. (NP304, C3, PS3c, Project No. 6034-22320-003-00D)

**Establishment of a new biological control agent of Cape-ivy.** Cape-ivy (*Delairea odorata* Lem.) is a vine-like perennial weed that has invaded sensitive coastal streambank, forest, and scrub habitats along

the California coast, and it is also invasive in Hawaii, Australia, and southern Europe. Cape-ivy smothers native herbs, shrubs, and trees and can clog coastal streams that supply agricultural water. ARS scientists in Albany, CA, released a fly from Cape-ivy's native range in South Africa that makes tumor-like galls in Cape-ivy's shoot tips, which reduces shoot tip abundance and growth. The fly is the world's first biological control agent against Cape-ivy. Releases were conducted at 18 field sites in California between late 2016 and 2019. The fly has established large populations at four sites, including two California State parks, with gall abundance increasing 40-fold since early 2019. Continued dispersal and galling will decrease Cape-ivy's competitive advantage and invasiveness. (NP304, C2, PS2b, Project No. 2030-22000-027-00D)

Beneficial nematodes complete their first trip to space. In an exciting collaboration between ARS researchers in Byron, GA, and industry partners, beneficial insect-killing nematodes (small roundworms) were sent to the International Space Station in support of the goal of developing environmentally friendly methods to support long-term space travel. Beneficial nematodes, also called entomopathogenic nematodes, are alternatives to using broad-spectrum chemical insecticides and are also safe to humans and other nontarget organisms. They are used to control a wide variety of insect pests on Earth. The nematodes sent into space were capable of navigating through soil and killing insect pests. This was the first biological control experiment in space. The mission represents a look into the future where food crops will be grown in space. (NP304, C3, PS 3a, Project No. 6042-22000-023-00D)

Frequency of pathogen transmission by stink bugs elucidated. It has been well established that stink bugs can transmit damaging plant pathogens that cause significant decline in cotton seed and lint yield. But the ability of individual stink bugs to infect multiple cotton bolls in succession, and the frequency of this occurrence, had not been known. ARS researchers in College Station, TX, discovered that an

individual southern green stink bug can infect as many as five cotton bolls in succession. This discovery provides a basis for revising stink bug treatment thresholds, which are currently based simply on numbers of stink bugs per unit area but do not consider whether the bugs may be carrying and depositing pathogens.

## New methods to control invasive horticultural pests and pests of quarantine importance.

Alternatives to methyl bromide fumigation are urgently needed for treating economically important insect pests of harvested fruit. ARS scientists in Parlier, CA, developed a novel postharvest fumigation protocol to control codling moth that were infesting shelled walnuts packed in new fiberboard cartons. U.S. exports of shelled walnuts to Japan are valued at \$80 million annually. A quarantine protocol for trailers containing citrus using postharvest fogging was developed against Asian citrus psyllids. A fumigation protocol using phosphine was developed against bean thrips to retain U.S. market access for fresh citrus to New Zealand, valued at \$20 million annually. Phosphine fumigation protocols were also developed to control black widow spiders and spotted wing drosophila to retain U.S. market access for table grapes to Australia valued at \$180 million annually. This ARS research directly resulted in market retention or expansion and served as the basis for interaction between industry, USDA Foreign Agricultural Service, USDA Animal and Plant Health Inspection Service, and respective counterparts in other countries. (NP304, C4, PS4a, Project No. 2034-43000-040-00D)

Integrated West Nile virus (WNV) early warning surveillance system developed. West Nile fever/encephalitis is the most important mosquito-borne disease in the continental United States. The disease is caused by a flavivirus that is separated into distinct lineages, with lineage 1 (L1) and lineage 2 (L2) encompassing all WNV known isolates associated with human and veterinary disease. Currently, all known U.S. WNV isolates belong to L1. L2 isolates, usually found in sub-Saharan Africa, were

recently found in Europe and caused large human and equine WNV outbreaks. The invasive threat and risk of WNV L2 invading the United States is significant because recent evidence has demonstrated that North American mosquito species are competent vectors of WNV L2 isolates from Africa and Europe. ARS scientists in Greece associated with the European Biological Control Laboratory designed an integrated WNV early warning surveillance system specifically targeting the L2 strains. The system relies on detecting viral RNA in field-collected mosquitoes and screening sentinel chickens for WNV-specific antibodies. The surveillance system was successfully implemented and provided information on WNV mosquito circulation and enzootic transmission 1 month prior to human cases, thereby allowing for targeted and proactive vector control interventions. Knowledge of the WNV L2 ecology in Europe combined with optimized field-based surveillance systems and laboratory diagnostic tools can be applied to enhance early detection and early warning systems to control and reduce this emerging threat. (NP304, C3, PS3a and PS3b, Project No. 0212-22000-027-00D)

Genes identified in insects that infest stored food products. Understanding the factors that allow insects to colonize different types of stored products will ultimately lead to tactics that can reduce and prevent infestations. Using recently compiled insect genomes from Ag100Pest, the effort to sequence the top 100 agricultural pests in the United States, ARS scientists in Manhattan, KS, in conjunction with researchers from University of Memphis, Indiana University, and several other research institutions have discovered rapidly evolving gene families that allow insects to adapt to new food sources. These gene families include those involved in the ability to perceive and respond to volatiles from food resources as well as those involved in the ability to digest complex carbohydrates present in plant cell walls and starchy foods. The ability to make long-read sequencing libraries from small amounts of insect tissue has greatly expanded the number of insect genomes scientists now have available to them and will lead

to further insights into genetic adaptations that have allowed diverse lineages of insects to exploit stored commodities. (NP304, C4, PS4b, Project No. 3020-43000-032-00D)

Microalga as a promising nutritional supplement for honey bees. Feeding honey bees an artificial pollen substitute diet to support colony health during periods of reduced forage is a common management practice by beekeepers, but most substitute diets need improvement. Artificial diets may be deficient in essential macronutrients (proteins, lipids, prebiotic fibers), micronutrients (vitamins, minerals), and antioxidants. In an effort to improve artificial diets, ARS researchers in Baton Rouge, LA, evaluated the nutritional aspects of the microalga Arthrospira platensis (commonly called spirulina), finding that spirulina is rich in the essential amino acids and functional lipids commonly found in pollen. Nutritional physiology and microbiome evaluations of bees fed spirulina closely matched those of bees fed a natural pollen diet. The study results thus show that the alga has significant potential to serve as a pollen substitute or prebiotic diet additive to improve honey bee health. Results of the study were highlighted in the August 2020 edition of American Bee Journal. More broadly, adapting beekeeping and broader livestock management practices with microalgae feeds could contribute to achieving objectives outlined in the United Nations sustainable development goals related to food security, sustainable water management, reversal of land degradation, and halting biodiversity loss. The long-term aim of this research is to characterize and develop microalgae as a sustainable feed source for honey bees that can be augmented via biotechnology to improve bee nutrition and health. (NP305, C2, PS 2A, Project No. 6050-21000-015-00D)

Universal intelligent spray control system as a retrofit for conventional sprayers commercialized.

An intelligent spray technology developed by ARS researchers in Wooster, OH, effectively controls pest insects and diseases with significant reductions in pesticide waste to the environment; however, to

ensure that growers use this technology economically, it must be adaptable to conventional sprayers. To address this challenge, the researchers developed a universal intelligent spray system as a retrofit unit for conventional orchard sprayers. The retrofit unit was tested in 15 commercial nurseries, fruit and nut orchards, and vineyards in California, Ohio, Oregon, South Carolina, Tennessee, Texas, and Australia. Field tests demonstrate this new technology can provide pest and disease control that is as effective as conventional spray systems while reducing spray drift by up to 87 percent and ground loss by 90 percent. In addition, pesticide use was reduced by up to 85 percent, resulting in an annual chemical cost saving of \$812 per acre, depending on crop type. This cost reduction does not include reductions in labor and fuel costs. The technology was transferred to a commercial partner and a commercial product, "Intelligent Spray Control System" by Smart Guided Systems, LLC, was released to the market. Citrus, apple, grape, nursery, and pecan growers in the United States and other countries have started to upgrade their sprayers with the commercial product. The use of a new laser-guided intelligent spraying system is beneficial to the environment and saves growers money. The ability to retrofit conventional sprayers offers a sustainable and environmentally responsible approach to protecting crops. (NP305, C1, PS 1E, Project No. 5082-21620-010-00D)

Bee genomics reveal a genetic basis of colony defensive behavior. Breeding using genomic tools has not yet been adopted by the honey bee industry. In part, this is because some of the traits of highest interest for bee breeding are regulated by many genes, which makes it a challenge to characterize them. In addition, honey bees live as a colony, and many relevant honey bee traits are measurable only at the group level. Colony defensive behavior is an ideal example in which many of these complications are evident. This trait is of particular interest to stakeholders because having bee colonies that are overly defensive is undesirable from both management and public health perspectives. By investigating the genomic structure of this clearly identifiable behavioral trait and using a novel population of gentle

Africanized honey bees local to Puerto Rico, ARS researchers in Baton Rouge, LA, were able to identify a particular region in the genome that contributes to reduced colony defensive behavior. These findings provide a roadmap for the analysis of complex bee traits. (NP305, C2, PS 2A, Project No. 6050-21000-015-00D)

Pennycress as a cash cover crop promotes sweet corn sustainability. Commercial sweet corn production often results in substantial losses of nitrogen applied via fertilizer that end up in ground and surface waters as a pollutant. Growing a cover crop after sweet corn harvest to use excess leftover nitrogen could prevent this issue, but producers are reluctant to adopt this practice for economic reasons. ARS researchers in Morris, MN, in collaboration with University of Minnesota scientists, demonstrated that pennycress, which can double as an oilseed cover and cash crop, reduces the potential loss of leftover nitrogen from sweet corn production by about 42 percent. Moreover, the excess nitrogen that pennycress scavenges is enough to produce adequate pennycress seed yields without adding any additional fertilizer. Results of these studies are relevant to farmers, extension specialists, and crop consultants searching for cover crop options that are both economically and environmentally sustainable. (NP305, C1, PS 1A, Project No. 5060-21220-007-00D)

The National Genetic Resources Program (NGRP). The NGRP is responsible for acquiring, characterizing, preserving, documenting, and distributing to scientists, germplasm of all life forms important for food and agricultural production. In FY 2020, 203,922 samples were distributed to 279 foreign genebank/resources units; international agricultural research centers; U.S. and foreign commercial companies; and U.S. and foreign agencies and universities.

Site	Samples	Accessions	Requests	Countries
Cotton Collection (COT)	646	551	65	2
National Arid Land Plant Genetic Resources	040	331	0.5	2
Unit (PARL)	146	125	22	3
National Laboratory for Genetic Resources				
Preservation (NSSL)	293	282	21	1
National Small Grains Collection (NSGC)	31,883	24,818	545	37
Natl. Germplasm Repository - Corvallis	7.500		0.00	1.0
(COR)	5,693	2,726	889	10
Natl. Germplasm Repository - Davis (DAV)	5,708	3,140	236	8
Natl. Germplasm Repository - Geneva (GEN)	4,012	2,030	276	6
Natl. Germplasm Repository - Hilo (HILO)	168	109	42	5
Natl. Germplasm Repository - Mayaguez				
(MAY)	576	222	65	2
Natl. Germplasm Repository - Miami (MIA)	249	200	61	4
Natl. Germplasm Repository - Riverside (RIV)	230	213	11	4
	230	213	11	4
North Central Regional PI Station (NC7)	45,539	20,029	1,119	49
Northeast Regional PI Station (NE9)	7,057	4,186	190	22
Ornamental Plant Germplasm Center (OPGC)	157	153	18	3
Plant Genetic Resources Conservation Unit, Griffin, GA (S9)	28,678	18,857	772	36
Plant Variety Protection Voucher Collection (PVPO)	45	45	3	1
Potato Germplasm Introduction Station (NR6)	3,530	1,429	174	11
Rice Genetic Stock Center (GSOR)	3,342	2,246	103	9
Soybean Collection (SOY)	18,987	11,671	346	17
U.S. National Arboretum (NA)	220	190	91	4
US Nicotiana Germplasm Collection (TOB)	326	252	68	8
Western Regional PI Station (W6)	46,437	30,266	893	37
Total	203,922	123,740	6,010	279

# 3.7. Outreach Activities: Workshops, Field Days, Trainings/Demonstrations, and Stakeholder Presentations/Meetings



AL	Soil Dynamics Research Unit	The laboratory was invited to present at the 2020 Tennessee Valley Cover Crop Field Day about cover crop mixtures and management. Participants were ag consultants and growers. There were approximately 75 attendees.
AL	Soil Dynamics Research Unit	The laboratory was invited to present at an Alabama Agricultural Experiment Station's virtual field day about cover crop mixtures preceding corn. The presentation was recorded and posted to an Alabama Agricultural Experiment Station Facebook page. Currently, there have been more than 1000 views and more than 300 views of the presentation.
AL	Soil Dynamics Research Unit	The laboratory was invited to present at the 2019 Alabama Row Crops Short Course about Cover Crops for Peanut Production Systems. Participants were growers, industry reps, and other researchers. There were approximately 150 attendees.

AL	Soil Dynamics Research Unit	The laboratory was invited to present management information about cover crops to Alabama Farmer's Cooperative (AFC) representatives and local growers as part of an information meeting to participants about cover crops. The meeting was at a field site where AFC has a cover crop field demonstration. There were approximately 40 attendees
AL	Soil Dynamics Research Unit	The laboratory was invited to present at the 2020 Louisiana Agricultural Technology & Management Conference about Cover Crop Management. Participants were ag consultants, industry reps, and other researchers. There were approximately 150 attendees.
AL	Dale Bumpers Small Farms Research Center	Attended the Southern Sustainable Agriculture Working Group Conference and presented two posters: 1) organic compared with conventional systems for lamb production yield differences in performance in southeastern U.S. pastures and 2) estimating the value of parasite resistance in sheep.
AR	Poultry Production and Product Safety Research Unit	The laboratory had a presentation on the economic and environmental impacts from pasture Best Management Practices (BMPs) at the Beaver Watershed Alliance's Pasture Aerator and Discovery Farm field day. Presented to approximately 30 producers interested in implementing on-farm conservation practices.
AR	Poultry Production and Product Safety Research Unit	The laboratory had a video presentation on understanding soils to understand agroforestry systems at the Soil Survey for Soil and Water Quality's Applications in the Natural State meeting. Approximately 100 producers, professors, industry, stakeholders, and students attended.
AZ	Southwest Watershed Research Unit	The laboratory led a training session on the Automated Geospatial Watershed Assessment (AGWA) tool, along with University of Arizona collaborators. Participants learned how to use AGWA to assess erosion control after fires, design green infrastructure, and perform scenario planning and watershed assessments.
CA	Agricultural Water Efficiency and Salinity Research Unit	The laboratory gave a presentation to the Desert Managers Group on "Evaluating the Efficiency of Drywell for Enhanced Aquifer Recharge."

CA	Commodity Protection and Quality Research Unit	The laboratory presented data on control of navel orangeworm in tree nuts to the industry.
CA	Invasive Species and Pollinator Health Research Unit	The laboratory provided a one-hour presentation entitled "Plant Physiology, Biology and Ecology: Why Management Works" to a workshop for the East Bay Municipal Utility District. Approximately 40 individuals attended.
СО	Central Plains Resources Management Research Unit	The laboratory presented research results to farmers and researchers at the Crop Production Conference.
DC	Floral and Nursery Plants Research Unit	The U.S. National Arboretum hosted a booth at the Mid-Atlantic Trade Show (MANTS) in Baltimore, MD, where approximately 12,000 attendees from the nursery and allied industries gathered to view products from 900+ vendors. The outcome was meeting new and reconnecting with existing stakeholders and hearing about problems, challenges, and pressing needs of the industry.
DC	Floral and Nursery Plants Research Unit	Scientists from the U.S. National Arboretum gave two talks to growers and researchers at the Southern Nursery Association Research Conference in Baltimore, MD, entitled: 1) "The U.S. National Arboretum - conserving native plants for the American landscape," and 2) "Molecular Variation of <i>Osmanthus armatus</i> hybrids."
FL	Citrus and Other Subtropical Products Research Unit	The laboratory presented research finding to University of Florida's Indian River Research and Education Center.
FL	Subtropical Horticulture Research Unit	The laboratory gave a presentation via Zoom to farmers in Puerto Rico and the Dominican Republic as well as an employee of a government agriculture organization of the Dominican Republic. The presentation gave diagnostic and management information for diseases found on cacao in Puerto Rico, with a focus on a virus recently found on the island.
IA	Agrosystems Management Research Unit	The laboratory gave a presentation at the Field to Market (FtM) General Assembly, during a concurrent session to update FtM members on the FtM Science Advisory Council and its activities. Presented on research challenges to updating the Water Quality component of FtM's Sustainability Metric.

IA	Agrosystems Management Research Unit	The laboratory gave the virtual presentation "Supporting NRCS use of the Agricultural Conservation Planning Framework in Priority National Water Quality Initiative Watersheds" to the Iowa Chapter of the Soil and Water Conservation Society during the chapter's summer meeting.
IA	Agrosystems Management Research Unit	The laboratory gave the presentation "Practices to Reduce the Loss of Nutrients and Sediment Through Tiles: Tile Inlet Filters, Bioreactors, and Saturated Buffers" at the North Central Iowa Crop and Land Stewardship Clinic, Iowa Falls, Iowa. Producers and extension specialists were in attendance.
IA	Agrosystems Management Research Unit	A laboratory scientist was a speaker and panelist at a workshop on "Connecting Soil Health and Watershed Health" which was hosted by the Foundation for Food and Agricultural Research (FFAR) on "Linking Soil and Watershed Health to In-field and Edge-of-Field Water Management" in Morgantown, WV.
LA	Honey Bee Breeding, Genetics, and Physiology Research Unit	In conjunction with the Louisiana Beekeepers Association, the Baton Rouge Bee Lab hosted its 23rd annual Field Day. Sessions were offered for beginning, intermediate, and advanced beekeepers with topics such as bee biology, basic equipment, honey bee nutrition, managing colony defensiveness, colony evaluation, swarming, splits, managing varroa, and queen rearing and selection.
LA	Honey Bee Breeding, Genetics, and Physiology Research Unit	The laboratory participated in a webinar for Apiculture Online: Hive Chat with North Carolina State University. The event was live streamed on YouTube. A few hundred beekeepers viewed the 1 hour stream. The event can be seen at https://www.youtube.com/watch?v=uvb6ZmZzcCM
LA	Sugarcane Research Unit	The laboratory had a presentation on weed control research at the Louisiana Agricultural Technology & Management Conference in Marksville, LA. The meeting was attended by approximately 50 scientists, industry personnel, and crop consultants. The presentation title was "Itchgrass and Italian ryegrass management."

LA	Sugarcane Research Unit	The laboratory had a presentation on its research at the American Society of Sugar Cane Technologists Meeting - Louisiana Division in Baton Rouge, LA. The meeting was attended by approximately 50 scientists, sugarcane producers, and industry personnel from Louisiana, Florida, and Texas. The presentation title was "Effect of burning postharvest residue on weed seed mortality after sugarcane harvest."
LA	Sugarcane Research Unit	The laboratory had a presentation on its research at the Extension/Research Sugarcane Training Meeting in Houma, LA. The meeting was attended by approximately 25 scientists, members of the American Sugar Cane League, and Louisiana State University County Extension agents. The presentation title was "Paraquat plus residual herbicides for managing itchgrass in plant-cane."
MD	Animal Genomics and Improvement Laboratory	The laboratory participated in an International Committee for Animal Recording Functional Traits Working Group meeting to discuss international standards for data recording and genetic evaluation.
MD	Animal Genomics and Improvement Laboratory	The laboratory participated in the National Dairy Herd Information Association Board Meeting in Rosemont, IL, to discuss industry research needs.
MD	Animal Genomics and Improvement Laboratory	The laboratory participated in a conference call with the American Angus Association team about selection goals.
MD	Animal Genomics and Improvement Laboratory	The laboratory attended the annual meeting of the National Dairy Herd Information Association and gave an invited presentation on its research.
MD	National Agricultural Library (NAL) Digitization and Access Branch	Presented talk on the life and collection of J. Horace McFarland, printer, horticulturalist, writer, and conservationist.
MD	NAL Information and Customer Services Branch	Provided multiple training to various USDA agencies (ARS, FAS, USFS, APHIS, NIFA, FNS, AMS, NRCS and FFAR) on DigiTop and NAL resources (journals, databases, news sources), services (document delivery), and EndNote reference management software.

MD	NAL Information and Customer Services Branch	NAL and Nutrition.gov teamed provided outreach with information, answering questions, demonstrations, and distributed information materials to attendees at the Public Libraries Association Conference 2020.
MD	NAL Information and Customer Services Branch	Completed multiple workshops and webinars remotely on Meeting the Information Requirements of the Animal Welfare Act to various stakeholders (Centers for Disease Control, Food and Drug Administration, Purdue University, University of Tennessee, Naval Medical Center, and American Association for Laboratory Animal Science).
MD	Natural Resources and Sustainable Agricultural Systems	The laboratory presented on current ARS research on ammonia emissions from agricultural activities at the Fall Meeting of the National Atmospheric Deposition Program. There were about 70 people in the audience from Federal agencies, State agencies, and universities.
MD	Natural Resources and Sustainable Agricultural Systems	The laboratory presented a virtual talk on its research at the Conservation and Drainage Network meeting. There were 115 participants from universities, private groups, and other Federal agencies.
MD	Natural Resources and Sustainable Agricultural Systems	Laboratory gave the presentation "Tying it All Together for Innovation in Conservation" at the National Association of Conservation Districts Annual meeting to 125 attendees.
MD	Natural Resources and Sustainable Agricultural Systems	Laboratory gave a presentation to 5,000 industry participants at the Environmental Systems Research Institute (ESRI) Fed Conference on Partners in Data Innovation.
MI	Sugarbeet and Bean Research Unit	Laboratory participated in an online virtual field day for the research station where trials are conducted. Michigan State University Extension organized, took video, and ran the event.
MN	Soil Management Research Unit	Laboratory gave an invited presentation on soil health at the Minnesota Seeding Contractors Association's Annual Meeting held in St. Cloud, MN.

MN	Soil Management Research Unit	Laboratory conducted an online presentation and demonstration on soil health for the Main Street Project's Climate Land Leaders. The title of the presentation was "What is soil health and how can we measure it?"
MN	Soil Management Research Unit	Laboratory gave an invited presentation entitled "The low-down on soil amendments" and gave a hands-on demonstration of earthworm dynamics at the 2019 Conservation Tillage Conference held in St. Cloud, MN. The event was sponsored by the University of Minnesota Extension.
MT	Range and Livestock Research Unit	ARS researchers presented research topics to the local producers and landowners. The range tour was coordinated by the MSU Extension Prairie County office.
MT	Range and Livestock Research Unit	Laboratory invited by the MT Red Angus Association to present "Are there other management practices I can effectively use for young cow reproduction?" during two sessions at the MT Stockgrowers Association's Mid-winter meeting.
NC	Plant Science Research Unit	Laboratory presented summary of modern breeding methods to Environmental Protection Agency and American Seed Trade Association meeting on plant breeding technology.
ND	Healthy Body Weight Research Unit	Laboratory participated in a roundtable discussion with University of North Dakota graduate students in nutrition about working in nutrition research, particularly human nutrition research.
NM	Range Management Research Unit	Laboratory conducted multiple Virtual LandPKS Training Part II for EcoCycle Community Carbon Farming Coordinators, Grameen Foundation Project, South Dakota Ranchers (BeefSD program), Stonyfield milk suppliers, and USAID.
NM	Range Management Research Unit	Laboratory held a Field Day focused on "Novel strategies for sustainable livestock production in arid rangelands." Field day highlighted collaborative research on the use of heritage cattle types, precision technologies, and decision-support tools that are aimed at sustaining production and minimizing costs and the environmental footprint of beef production in arid lands.

NM	Range Management Research Unit	Laboratory gave the keynote address on climate change adaptation and social marketing at the NM Farmers Marketing Association. Also curated two Science on a Sphere presentations.
NM	Range Management Research Unit	Laboratory presented in a webinar series for the Bureau of Land Management's Assessment, Inventory, and Monitoring (AIM) project leads and data users that described the process of using AIM data for ecological site development.
NM	Range Management Research Unit	Laboratory held a virtual workshop at Grassland Expo for front range ranchers open space managers.
ОН	Application Technology Research Unit	Laboratory hosted a demonstration for 72 Ohio Farm Bureau staff and leadership members on how the intelligent sprayer technologies can reduce pesticide use, increase production profits, and safeguard the environment for growers.
ОН	Application Technology Research Unit	Laboratory gave a presentation on weed control for landscapes and field nurseries at the 2020 Kentucky Nursery and Landscape Association Educational Outing and Expo in Louisville, KY.
ОН	Application Technology Research Unit	Presented Virtual Grower at the Local Agricultural Marketing Program's annual meeting in Riverhead, NY
ОН	Soil Drainage Research Unit	Laboratory gave a presentation titled "USDA-ARS Edge-of-Field Research: Results and Future Direction" at Science and Solutions meeting in Fort Wayne, IN. Attended by 32 State and Federal agencies and university and Lake Erie stakeholders.
ОН	Soil Drainage Research Unit	Laboratory gave presentation titled "Finding the Balance between Sacred Cows and Sacrificial Lambs (Environmental vs Agronomic Practices)" to an audience of 30 policy, extension, practitioner, and NGO stakeholders.
ОН	Soil Drainage Research Unit	Laboratory gave a presentation titled "USDA-ARS, Edge of Field Research in Ohio" to approximately 60 state and national agency and Great Lake's stakeholder representatives at the annual Great Lakes Regional Sediment Management Workshop, at the Army Corp of Engineers offices in Chicago, IL.

ОН	Soil Drainage Research Unit	Laboratory gave a presentation on the impacts of drainage water management on addressing water quality issues to more than 75 extension educators, producers, researchers, and Lake Erie stakeholders at the Conservation Tillage and Technology Conference (Ada, OH).
ОН	Soil Drainage Research Unit	Laboratory gave a presentation on edge-of-field data to a group of NRCS, Ohio Soil and Water Conservation Districts, Farm Bureau, and other farming related group personnel at the Federation of Ohio Soil Water Conservation Districts annual meeting.
ОН	Soil Drainage Research Unit	Laboratory gave a presentation on what is more important for water quality, recently applied P or legacy soil P, at the Conservation Tillage Conference in Ada, OH.
ОН	Soil Drainage Research Unit	Laboratory gave a presentation titled "Research Update: Using Drones (UAVs) to Map Subsurface Drainage" at the virtual 2020 Conservation Drainage Network Annual Conference. This meeting was attended by university, government, and industry personnel.
ОН	Soil Drainage Research Unit	Laboratory gave a presentation titled "Research Update: Using Drone Aerial Imagery for Drainage Mapping" at the 2020 Ohio Land Improvement Contractors Association (OLICA) Annual Meeting in Dublin, OH
OK	Great Plains Agroclimate and Natural Resources Research Unit	Laboratory attended and participated in the Southwest Beef Symposium held in cooperation with New Mexico State University and Texas A&M University, which is part of the Sustainable Southwest Beef Cross Agency Project. Laboratory scientists interacted with participants (including ranchers, exhibitors and speakers), answered questions, and manned the exhibit table.

OK	Great Plains Agroclimate and Natural Resources Research Unit	Laboratory hosted the Long Term (LTAR) Regionalization and Unmanned Aerial Systems (UAS) Workshop. The group finalized development of the first manuscript to be produced by the Remote Sensing /Geographical Information Systems Working Group, had discussions concerning collaborative network-wide research projects, integration of remote sensing technologies into UAS platforms, the LTAR Data Innovations project, and a session on working with calibrated UAS data.
OK	Hydraulic Engineering Research Unit	Updated an activity book and developed a "how-to" soil erosion demonstration brochure to be sent to the American Society of Agricultural and Biological Engineers to be used for their social media during distance learning.
OK	Hydraulic Engineering Research Unit	Laboratory hosted an internal erosion workshop.
OR	Horticultural Crops Research Unit	Laboratory was invited to talk about its research program to members of the Idaho State Horticultural Society.
TX	Cotton Production and Processing Research Unit	Laboratory presented on the best practices for safe seed cotton storage in round modules and detection and elimination of plastic contamination in seed cotton at the Red River Crops Conference in Altus, OK. This conference brings together cotton producers and researchers from Texas, Oklahoma, and Kansas to discuss key issues faced by the cotton and grain industries in the region.
TX	Cotton Production and Processing Research Unit	Laboratory presented on cotton fiber quality and detection and elimination of plastic contamination from seed cotton in the ginning process at Cotton Incorporated's Great Plains Cotton Conference in Wichita, KC. This meeting brought together cotton producers and researchers from Texas, Oklahoma, Kansas, Tennessee, North Carolina, and California to discuss issues faced by the cotton industry in the northern-most growing region of the US.
UT	Poisonous Plant Research Unit	Laboratory had a booth at the Utah Cattlemen's Association Trade Show.
UT	Poisonous Plant Research Unit	Laboratory participated in the Converse (Wyoming) County Weed & Pest Growers Annual Meeting.

WA	Physiology and Pathology of Tree Fruits Research Unit	Laboratory presented its current postharvest apple research at the Washington Tree Fruit Research Commission research review.
WA	Physiology and Pathology of Tree Fruits Research Unit	Laboratory described results from its recent research on postharvest biology of pear fruit at the Pacific Northwest NW Pear Committee annual research review in Yakima, WA. Presentations described how new techniques could support enhanced fruit quality and reduce losses after long-term cold storage of pear fruit.
WA	Physiology and Pathology of Tree Fruits Research Unit	Laboratory gave presentations describing its recent research at the Washington State Fruit Association Annual Meeting.
WA	Temperate Tree Fruit and Vegetable Research Unit	Laboratory participated in Washington State University's Prosser's Centennial celebration and educated community members and local students on the important research being conducted by its scientists, specifically teaching them about the potato breeding and pathology programs.
WI	Vegetable Crops Research Unit	Laboratory presented its research results to potato farmers.
WI	Vegetable Crops Research Unit	Laboratory performed two hands-on workshops focused on its research to improve potato genetics. The workshop discussed potato cultivation in general, and led hands-on experiments to determine the effect of increased sugar content in potatoes on fry color and the identification of virus-infected plants using commercially available test strips.
WV	Innovative Fruit Production, Improvement and Protection Unit	Laboratory presented its research on the invasive spotted lanternfly to the West Virginia Invasive Species Working Group that includes State Dept of Agriculture personnel, regional IPM, and university specialists.
WV	Innovative Fruit Production, Improvement and Protection Unit	Laboratory taught a grafting workshop at the Shepherd University Agricultural Innovation Center at Tabler Farms in Shepherdstown, WV. The goal was to educate the participants on the uses of grafting and why it is important in horticulture. They can now graft fruit trees in their own back yards or farms after this workshop.

WV	Innovative Fruit Production, Improvement and Protection Unit	Laboratory taught a pruning workshop at the Air Guard Base in Charleston, WV. The goal was to educate the participants on how to prune and maintain fruit trees, mainly apple trees in their own back yards or farms
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## 3.8. FY 2019 Technology Transfer Award Winners

## **ARS Technology Transfer Award**

Scientists: Terry Arthur and Tommy Wheeler, Meat Safety & Quality Research Unit (Clay Center, NE)

**Title:** Beef Trim Sampling Methods Team

## Federal Laboratory Consortium for Technology Transfer (FLC) Awards

Laboratory: Coastal Plain Soil, Water and Plant Conservation Research Unit (Florence, SC)

Title: Odor/Ammonia Capping of Swine Lagoons using High Performance Nitrifiers

Award: National, Excellence in Technology Transfer

**Laboratory:** Soil and Water Management Research Unit (Bushland, TX)

Title: Sensor Based Variable Rate Irrigation Control Increases Crop Water Productivity

Award: National, Excellence in Technology Transfer

**Laboratory:** Soil and Water Management Research Unit (Bushland, TX)

Title: Sensor Based Variable Rate Irrigation Control Increases Crop Water Productivity

Award: National, Technology Focus Award

**Laboratory:** Office of Technology Transfer (Beltsville, MD)

**Title**: Innovation Fund Grant Program

Award: National, Technology Transfer Innovation Award

Person: Robert J Griesbach

Award: National, Harold Metcalf FLC Service Award

Person: Robert J. Griesbach

Award: Mid-Atlantic Region, Outstanding Technology Transfer Professional

**Laboratory:** Produce Safety and Microbiology Research Unit (Albany, CA)

Title: Reversible Antimicrobials: A Strategy to Reduce Ecotoxicity and Antibiotic Resistance

Award: Far-West Region, Outstanding Technology Development

**Laboratory:** Soil and Water Management Research Unit (Bushland, TX)

Title: Sensor Based Automatic Variable Rate Irrigation Control for Greater Crop Water Productivity

Award: Mid-Continent Region, Excellence in Technology Transfer

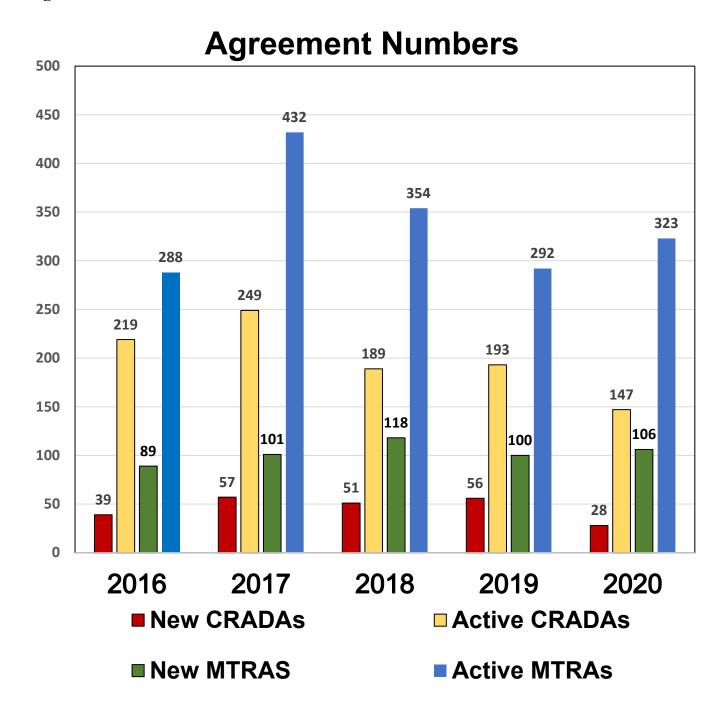
Laboratory: Coastal Plain Soil, Water and Plant Conservation Research Unit (Florence, SC)

Title: Odor and Ammonia Capping of Swine Lagoons Using High-Performance Nitrifiers

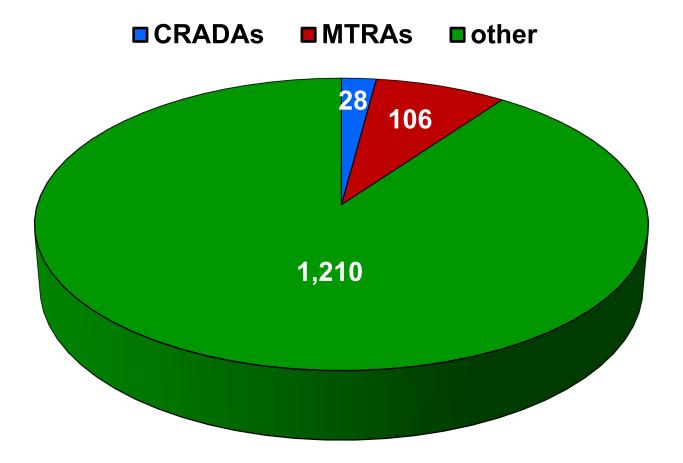
Award: Southeast Region, Excellence in Technology Transfer

## 3.9. Selected Metric Charts.

Figure 1. Number of new and active CRADAs and MTRAs.



**Figure 2.** Number of collaborative research agreements (CRADAs, MTRAs, and other agreements, including Trust Fund Cooperative Agreements, Reimbursable Agreements, Interagency Agreements, and Non-Funded Cooperative Agreements) executed by type in FY 2020.



**Figure 3.** Number of invention disclosures, patent applications filed, and patents issued. The year in which a patent issues is not the year in which the patent is filed. The increase in the number of invention disclosures in FY 2018 was the result of a significant increase in biological materials disclosures.

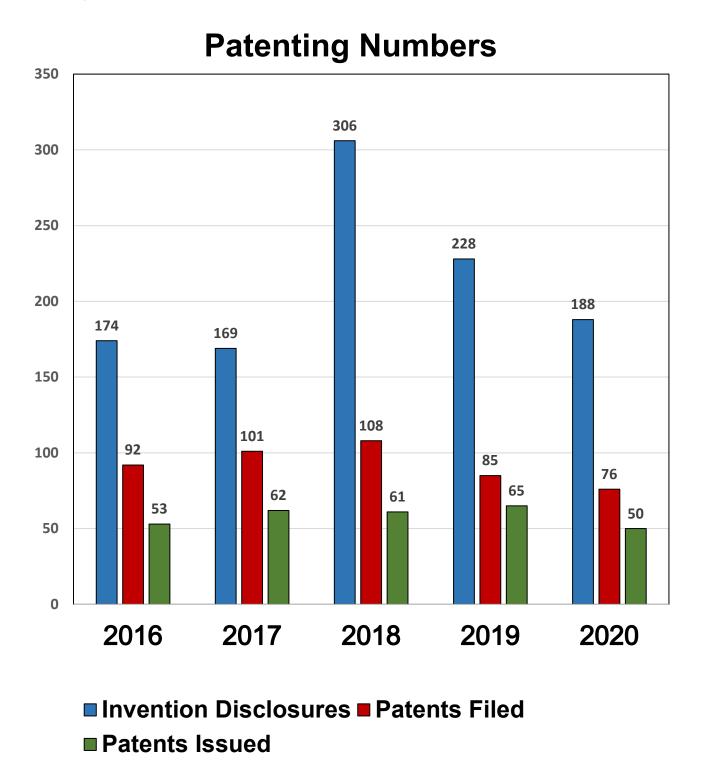
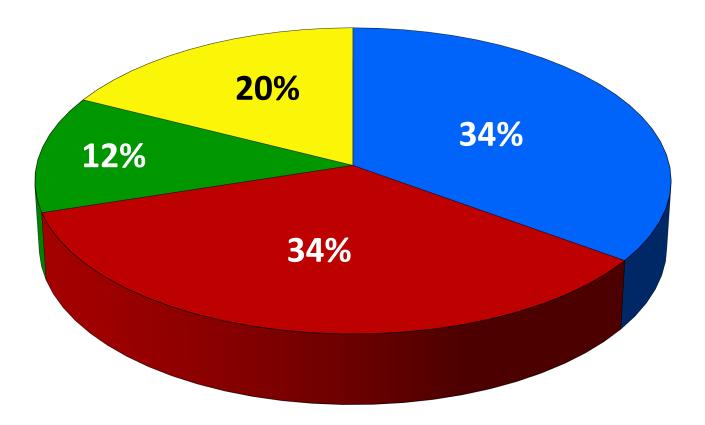


Figure 4. Percentage of patents issued in FY 2020 by scientific discipline.





**Figure 5.** The ratio of patents issued over patent applications filed per year. Although the year in which a patent is issued is not typically the year in which the patent application is filed, over time the ratio of patents issued over the number of patent applications filed is an indicator of "judicious" patenting. Over the last 5 years, this indicator suggests that  $\sim$ 62 percent of the patent applications result in an issued patent.

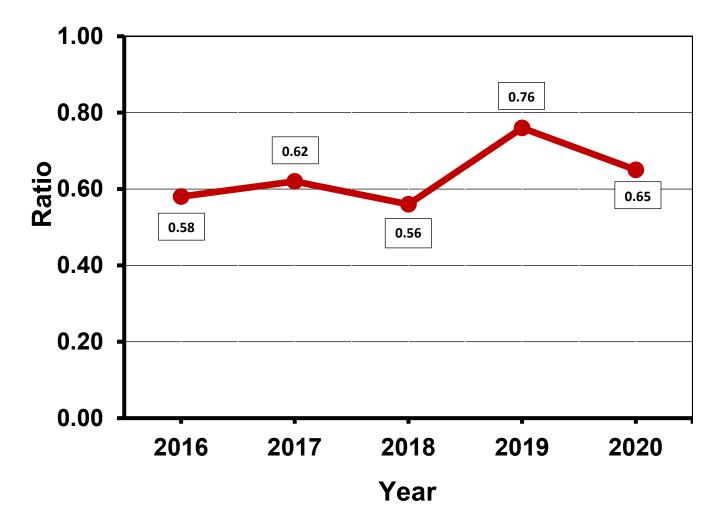
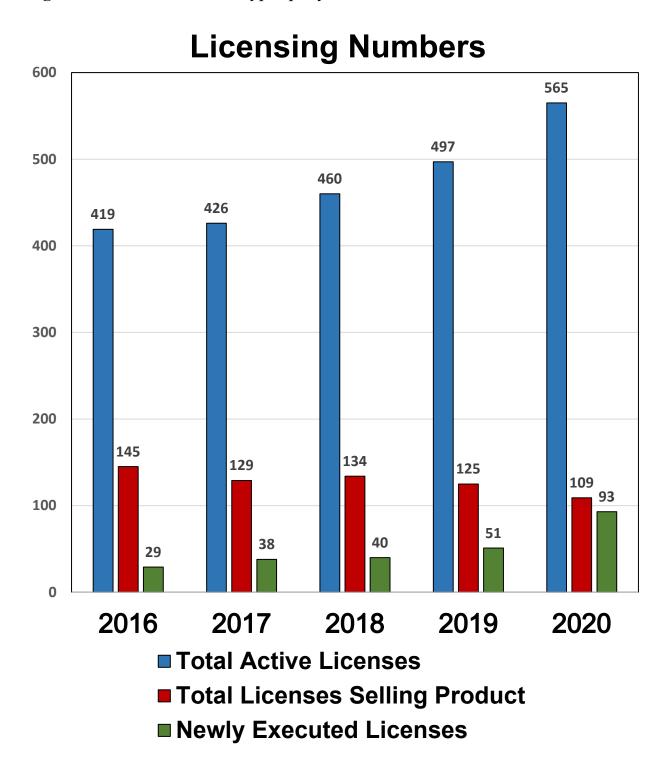


Figure 6. Number of license types per year



**Figure 7.** Earned license royalty income (ERI) over time. The lower income in FY 2018 and FY 2020 was due to several of the top revenue generating licenses expiring in FY 2017 and 2019.



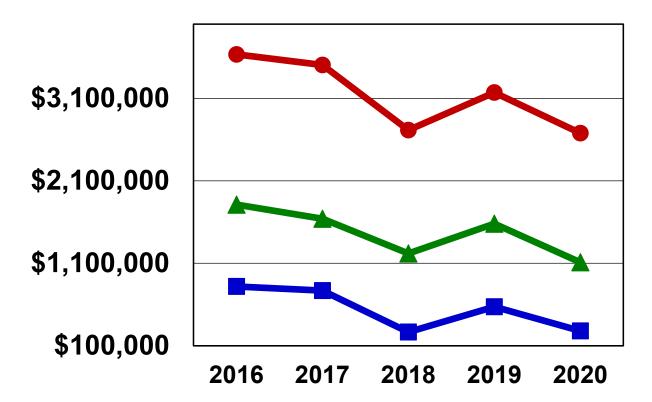
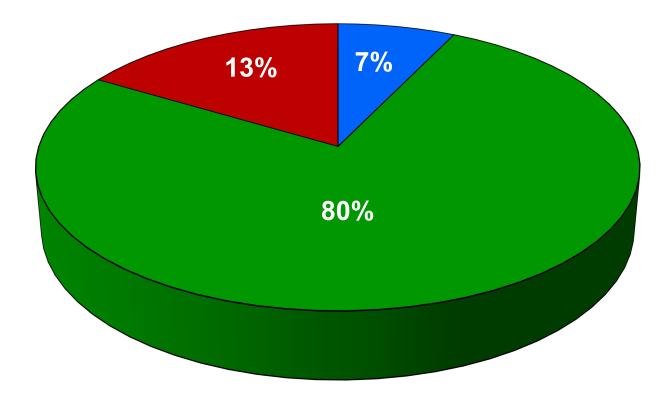


Figure 8. The percentage of new licenses executed in FY 2020 by business type.

■ Universities■ Small Businesses■ Medium & Large Businesses



**Figure 9.** The ratio of newly signed invention licenses over newly issued patents per year. Although the year in which a license is signed is not typically the year in which the patent has issued, over time the ratio of newly signed licenses over the number of newly issued patents is an indicator of "judicious" patenting contemplating among other things commercial viability of the technology.

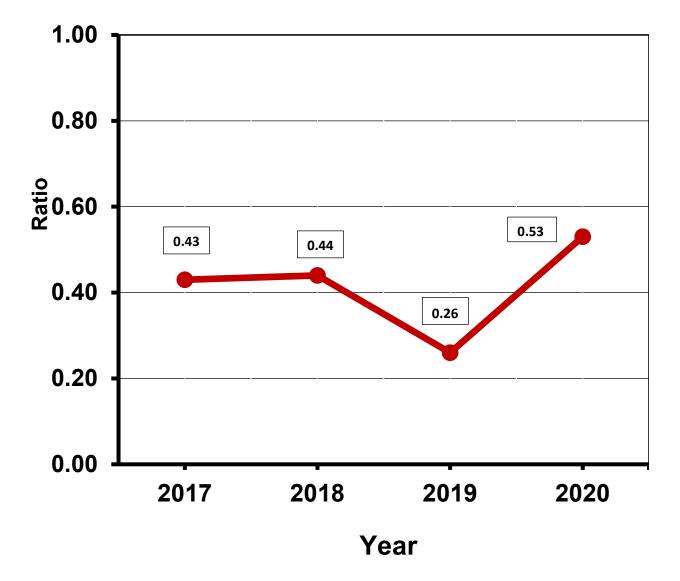


Figure 10. Number of publications per year

