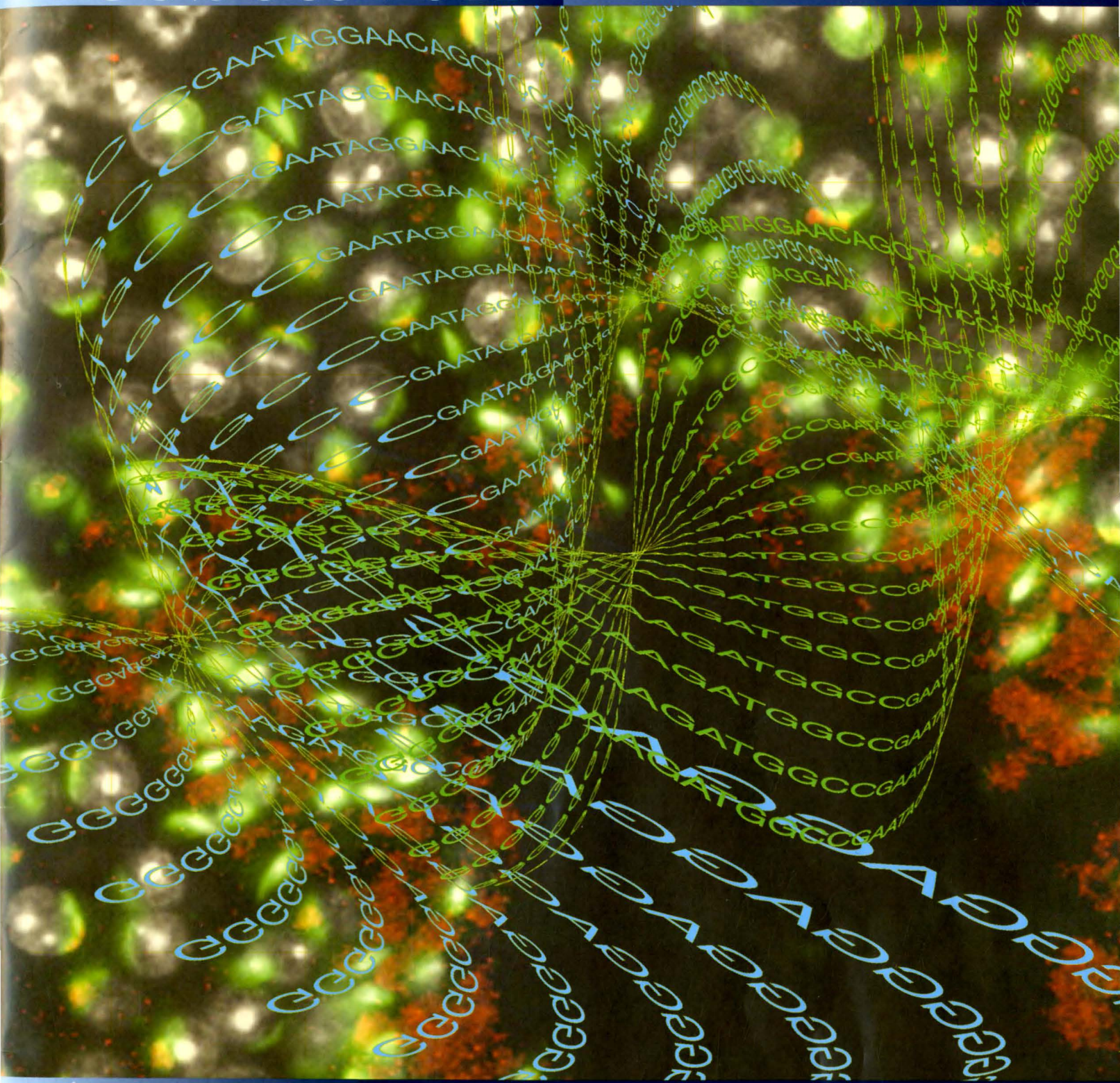
Research

SOUTH DAKOTA STATE UNIVERSITY



South Dakota State University

SDSU Spring 2016

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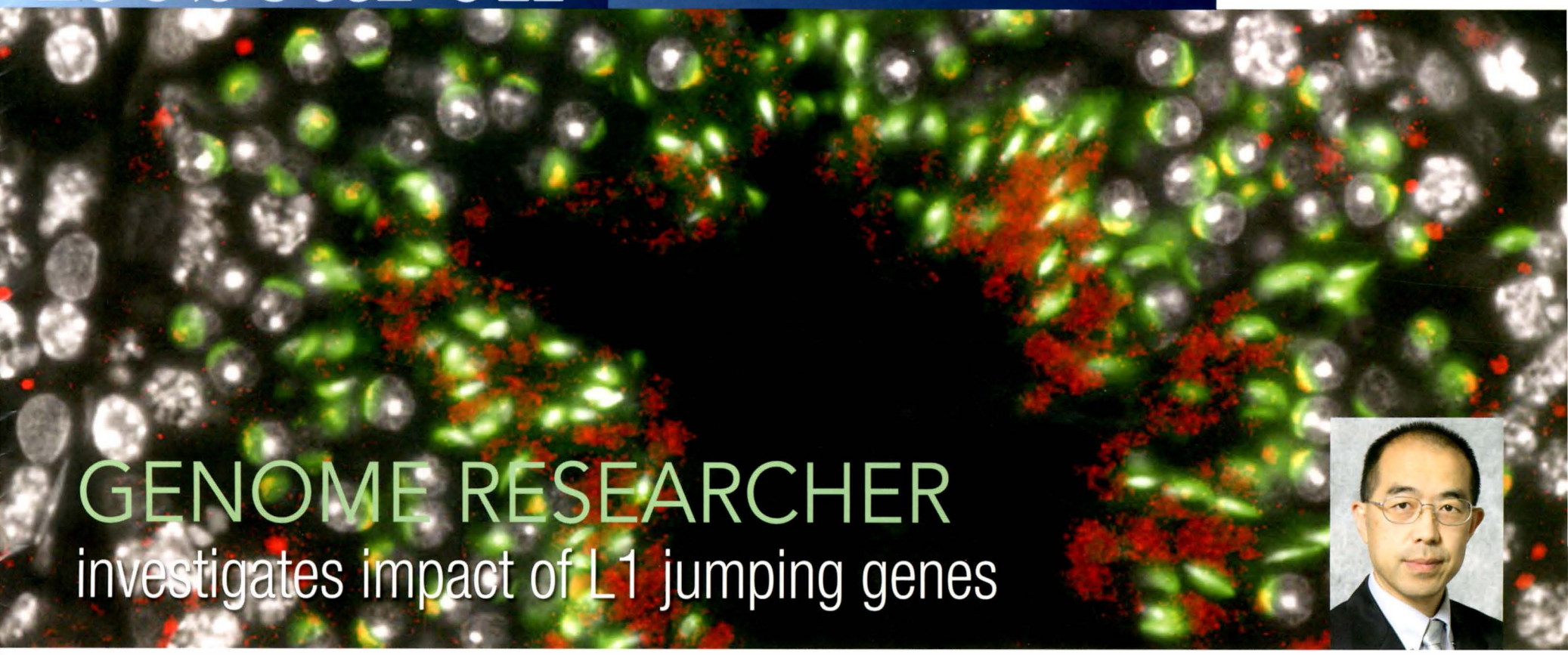


GENOME RESEARCHER
investigates impact of L1 jumping genes

Wenfeng An

They're called jumping genes, the DNA segments that are able to replicate themselves to a new position on the same or another chromosome. These movable DNA segments, also known as transposable elements, contribute to genetic diversity and are a major driver of evolution, but sometimes these mutations can lead to dysfunction and disease, according to associate pharmaceutical sciences professor Wenfeng An.

Typically genes are static, An explained. "They reside in the genome, will be expressed and carry certain functions." These transposable elements, which were once thought of as useless, can affect the way in which genes are expressed. Jumping genes exist in humans, animals, plants, insects and even yeast.

An and his research team are investigating how a particular type of jumping genes, long interspersed elements type 1, known as L1, causes mutations within body and reproductive cells. His lab is part of the Center for Systems Biology of Retrotransposition funded by the National Institute of General Medical Sciences.

Since moving his lab to South Dakota State in November 2014, An has secured two two-year National Institutes of Health grants in June 2015 for nearly $800,000 and, by 2018, will be receiving a total of about $1.4 million in federal funding. An, who has worked with L1s since 2003, is the College of Pharmacy's first endowed scholar, a cancer research position created through a trust established by SDSU alumni Barry and Sharon Markl.

L1 jumping gene abundance

About half of human DNA is made up of a variety of repeated segments, but L1 segments constitute the largest portion—17 percent of the human genome. L1s are littered throughout the human genome, An explained. "If you think of the genome as a book written in DNA alphabet, L1s are like a phrase that comes up again and again. You can find them quite a few times on every single page of the book."

An describes the replication of L1 segments as a "copy and paste mechanism." The original DNA segment remains but the copy is transcribed to RNA, a simplified version of the original, and then converted back into DNA before being inserted somewhere else within the genome.

Normally the cells of an organism exert tight control over this mechanism, but during what An called "certain developmental time windows," such as when neurons are being differentiated in a fetus, these controls are relaxed. "There's a lot of remodeling going on," he added. "This is also the time frame in which these jumping genes tend to have higher activity."

L1 insertions leading to disease

Where these L1 segments land within the genome determines what, if any, effect they have, An explained. "When they land inside a gene, they may inadvertently shut down its normal function."

An estimated 80 percent of the human genome can be assigned to a biochemical function, he pointed out. Consequently, "the chance of disrupting a function is pretty high."

For instance, scientists have identified 18 cases of neurofibromatosis that can be traced to an L1-mediated insertion that disrupted the expression of the NF1 gene, An pointed out.

The genetic disorder causes tumors to grow on nerve tissue, according to the National Institute of Neurological Disorders and Stroke. NF1 is the most common type of neurofibromatosis, affecting 1 in 3,000 to 4,000 people in the United States; however, only 30 to 50 percent of new cases can be traced to "a spontaneous genetic mutation of unknown cause."

In the case of disease development, such as cancer, control can be lost. Other research studies have shown that L1 insertions can inactivate tumor-suppressing genes or activate oncogenes, contributing to cancer formation.

Using next-generation sequencing, researchers have identified many new L1 copies present in cancer cells but not in the patient's normal tissue cells, according to An. Other research groups have identified colorectal, lung and head and neck cancers, in particular, as having increased L1 insertion rates.

This may open the door for prevention strategies aimed at inhibiting L1 activity; however, An cautioned, a better understanding of L1 activation during cancer progression requires significant research efforts, which have been currently hindered due to lack of suitable mouse models. That's one of the challenges that he hopes to overcome.

Improved mouse model

An is using a transgenic mouse model—mice that carry an introduced L1 DNA but otherwise are genetically identical—which then allows the researchers to identify any genetic changes as being specifically caused by the L1 transgene in body and reproductive cells, specifically sperm cells.

"The mouse model will allow us to observe L1's impact on the entire process of animal development, which can't be fully replicated in a cell-based model." However, he added, "We are not the first group to develop an L1 mouse model."

Two aspects of the research projects are unique. First, An and his team of one postdoctoral research associate, three doctoral students and three undergraduates, are utilizing mouse L1-based transgenes, rather than human L1s. The L1 transgene is injected into single-cell mouse embryos to make the transgenic mouse line, An explained. "We have preliminary experimental evidence to suggest that only the mouse-derived L1 transgene is regulated transcriptionally similar to how the endogenous L1s are regulated in mice."

Second, the researchers are using a single copy transgene, rather than tandem copies. In addition, the L1 transgene is molecularly tagged to facilitate identifying the inserted L1 copies. The researchers can also measure how frequently the insertions occur by using a highly sensitive droplet digital polymerase chain reaction, or ddPCR, approach.

The NIH grant, funded through the Office of Research Infrastructure Program, supports development and characterization of the novel L1 mouse models to understand how L1 insertions function under a controlled or regulated environment and during developmental time frames when those controls are relaxed or unregulated.

L1 role in birth defects

The other NIH grant, from the National Institute of Child Health and Human Development, focuses on the role L1 insertions play in birth defects. An and his research team will look at the impact of paternal aging on L1 insertions by evaluating the frequency of L1 insertions in sperm cells of mice at ages equivalent to a 20-, 30-, 42- and 56-year-old human father.

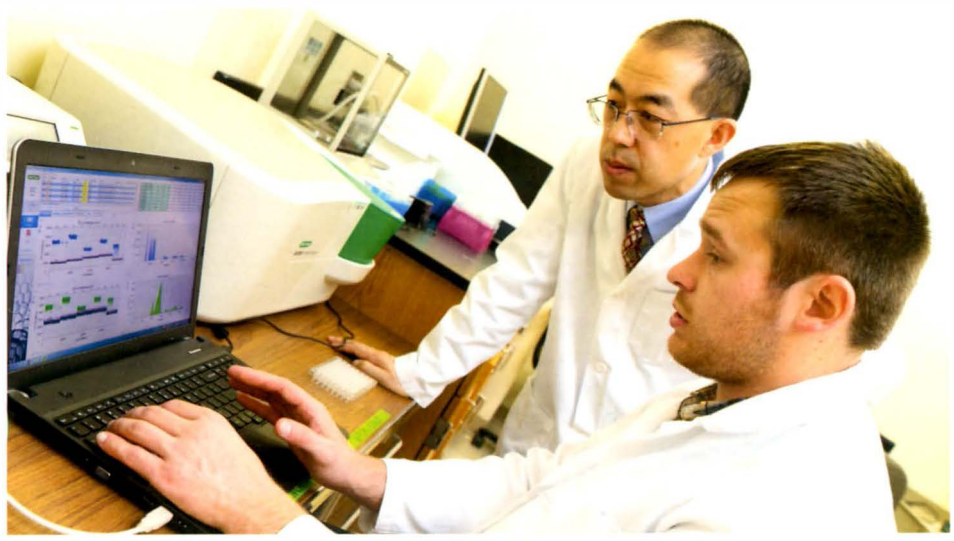
The researchers will test the hypothesis that L1 activities are developmentally regulated in the male germline and that the frequency of L1 insertions increases as males age. An said, "We are looking at whether L1 mediated insertional mutagenesis plays a role in major birth defects and, in particular, what plays a role in the so-called paternal effect."

An and his team will plot the L1 insertion frequency compared with paternal mouse age. "If we see a linear increase, that will correspond to a weak age effect," he said. "If the frequency is higher than expected in advanced age, possibly even an exponential increase, that would be a strong age effect."

Thus far, no one has been able to prove or refute the L1 and paternal age association hypothesis, but An is optimistic: "We should be able to get a clean answer."

The long-term goal is to moderate the adverse effects created by abnormal L1 activities, An explained. "The data we get from the mouse models will provide important guidance regarding what types of cells and processes we should look at in human individuals and what kinds of precautions we can take in order to ameliorate the risk of L1 jumping genes."

Above: This cross section of seminiferous tubules in a mouse testes shows the L1 protein in red, the cell nucleus in white and the spermatids in green. Sperm cells develop in tubules that loop through the testes.
Below: Associate professor Wenfeng An and doctoral student Simon Newkirk review results from the droplet digital polymerase chain reaction (ddPCR), which can quantify DNA targets down to one copy in 100,000 cells.



Creativity leads to innovations, inventions

"Creativity is thinking up new things. Innovation is doing new things."

This insight from American economist Theodore Levitt, a professor at Harvard Business School, encapsulates the land-grant mission that drives research at South Dakota State University. Our talented researchers use their knowledge, experience, imagination and creativity to develop innovations that address problems, such as combating diseases, enhancing fish and wildlife habitat and protecting the environment.

Geneticists are studying the behavior of transposable elements, movable DNA segments that can replicate themselves to a new position on the same or another chromosome. They are investigating how certain transposable elements cause mutations that trigger disease and cancer development.

Exercise and behavioral scientists are developing interventions that will help multiple sclerosis patients improve their quality of life, while nurse-researchers are encouraging health-care facilities to implement tobacco-free policies that motivate tobacco users to quit.

Protecting crops against diseases requires constant vigilance by our team of plant pathologists. They monitor and identify pathogens in South Dakota fields, develop management strategies and then help plant breeders develop cultivars with resistance to emerging pathogens.

Others are field-testing their innovations, bringing them closer to commercialization. Engineers are testing a cost-effective means of removing phosphorous from tile drainage water by adding a barrel loaded with steel shavings to a woodchip bioreactor near Baltic. This system will help reduce the environmental impact of drainage tiling and improve water quality in our streams and rivers.

In addition, natural resource management researchers hope one day to see the fish ladder they designed positioned on culverts across the state and nation. Their patent-pending invention reconnects the stream habitat for small-bodied fish, such as minnows, so they can move upstream to feed and spawn.

As part of the land-grant imperative and the entrepreneurial spirit that Levitt encourages, dedicated scientists and engineers at South Dakota State develop innovations and inventions to improve the lives of this and future generations.

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important resource for hunters

Land set aside under the Conservation Reserve Program plays a key role in drawing out-of-state hunters to South Dakota. That's the take-home message from a U.S. Department of Agriculture Farm Service Agency hunter survey.

Nonresident hunters spent more than $122 million in the 2014 season with 89 percent of that coming from upland game or pheasant hunting. More than $37.5 million was generated through nonresidents who hunted on CRP land, with upland game hunting accounting for nearly 95 percent, or $35.45 million, of those monies, which researchers refer to as economic impact.

Economic impact looks at the money nonresident hunters spend on everything from gas to food and lodging as well as licenses and shells, explained assistant economics professor Michael Miller, who helped analyze the South Dakota data. The influx of "new money" would not have occurred if those hunters had not come to the state.

"We wanted to find out how much upland game, waterfowl and deer hunting depended on CRP land for two reasons. First, there is concern that CRP land is not being re-enrolled. Second, the perception exists that putting land into CRP, and thus, taking it out of agricultural production, adversely affects the local economy," said project manager John Loomis, a professor in the Department of Agricultural and Resource Economics at Colorado State University. Data was also collected on North Dakota hunters through the $250,000 research project.

CRP, which began in 1985 and is administered by the Farm Service Agency, allows producers to remove marginal, environmentally sensitive land from production in return for a yearly payment. The goal is to return the land to perennial grassland to improve water quality, prevent erosion and provide wildlife habitat.

Calculating economic ripple effect

Surveys were sent to 2,863 hunters who bought upland game, waterfowl and deer licenses for the 2014 season to assess the economic contribution, impacts and benefits of hunting in South Dakota, according to Miller. More than 39 percent of deer hunters, 35 percent of upland game hunters and 33 percent of waterfowl hunters returned their surveys.

Resident and nonresident hunters reported slightly more than $282 million in direct expenditures. About 62 percent of those dollars—$175.7 million— came from upland game or pheasant hunting. Deer hunting accounted for about 20 percent, $56.1 million, and waterfowl hunting was close behind with 18 percent, or $50.6 million.

However, those direct expenditures don't paint a complete picture of how those dollars reverberate within the state's economy, Miller pointed out. To do that, the researchers analyzed the data using IMPLAN software, which considers indirect effects and induced effects.

Loomis describes it as throwing a rock into a pond, "there's always that ripple effect." The envelope of money

Nurse-researchers

Health-care facilities being tobacco-free seems like a natural fit, but it's far more complicated than it might seem.

"To be tobacco-free, a facility must prohibit tobacco and smoking in buildings and on campus grounds it owns and leases. It must be enforced 24-7 and prohibit smoking or tobacco for all people," explained assistant nursing professor Heidi Mennenga.

That's a tall order, particularly when it comes to enforcement, she admitted. "From a hospital perspective, I can see that being difficult. Is it my job as a staff nurse or is that security's responsibility? And what are the consequences?"

Through a one-year, approximately $50,000 grant from the South Dakota Department of Health, Mennenga and a research team surveyed South Dakota health-

Electronic health record systems prompt the nurse to ask each patient about tobacco use.

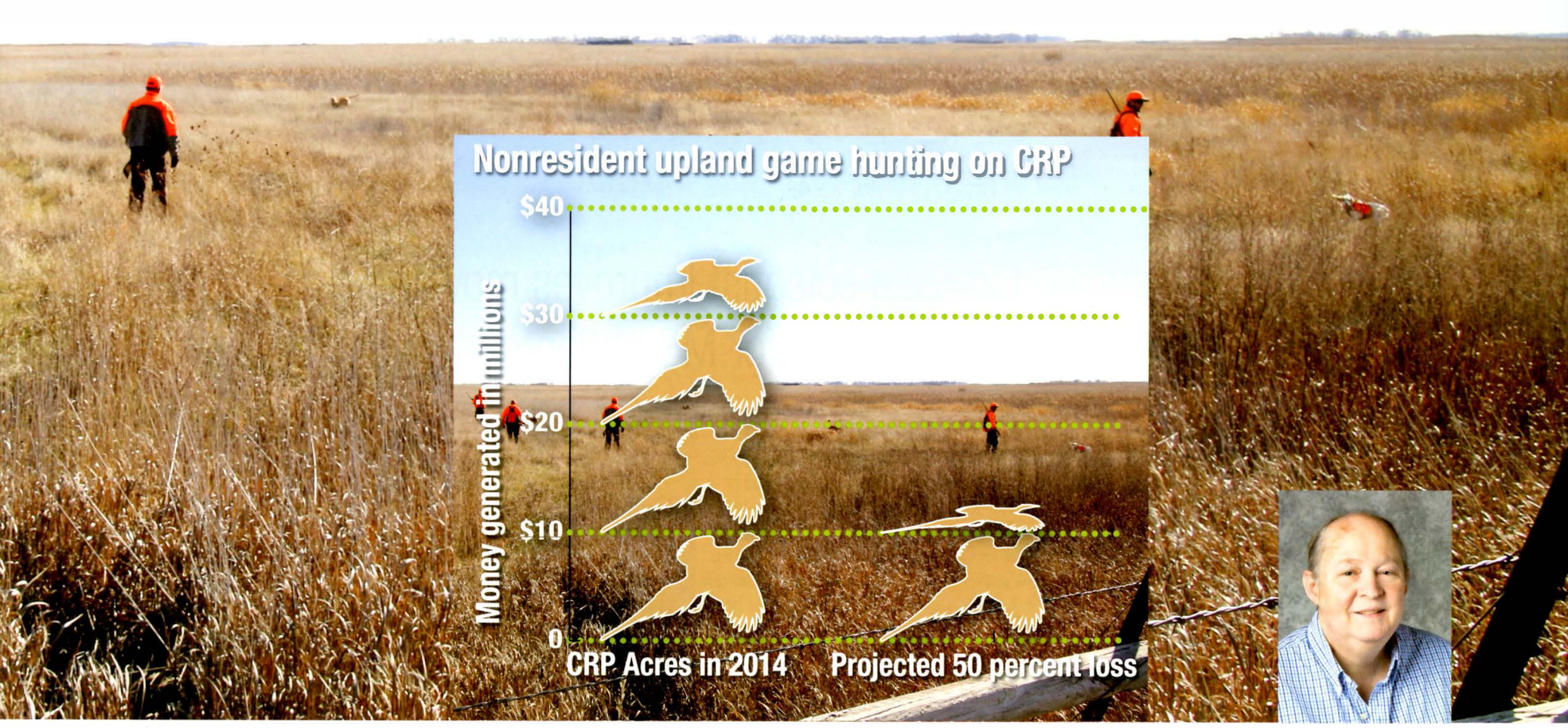


Photo courtesy of Tom Koerner, U.S. Fish and Wildlife Service

Michael Miller

that the hunter throws into the pond then ripples throughout other sectors of the economy.

The restaurants at which the hunters dine use those dollars to purchase supplies and to pay their employees. Those would be indirect effects, Miller explained. When those employees spend the money they earn for the things they need, those are induced effects.

"Eating at a restaurant, for instance, creates more ripples than buying sandwich meat and bread at a grocery store," Loomis explained. The model also accounts for whether a baker in South Dakota makes the bread.

"It's not a perfect model, but a very good one at linking the interconnectedness, how that initial envelope of money the hunter brings in ripples through other sectors," he said.

The analysis showed that the economic contribution from nonresident pheasant hunting alone brings more than $63 million in economic benefits, with $32.4 million in wages associated with approximately 1,300 jobs. When resident pheasant hunters are included, those figures increase to $87 million in economic benefits, $49 million in wages and 1,938 jobs.

Clearly, pheasant hunting is the most impactful, Miller said. Simply put, he pointed out, "we have the best pheasant hunting in the world."

Estimating impact of CRP losses

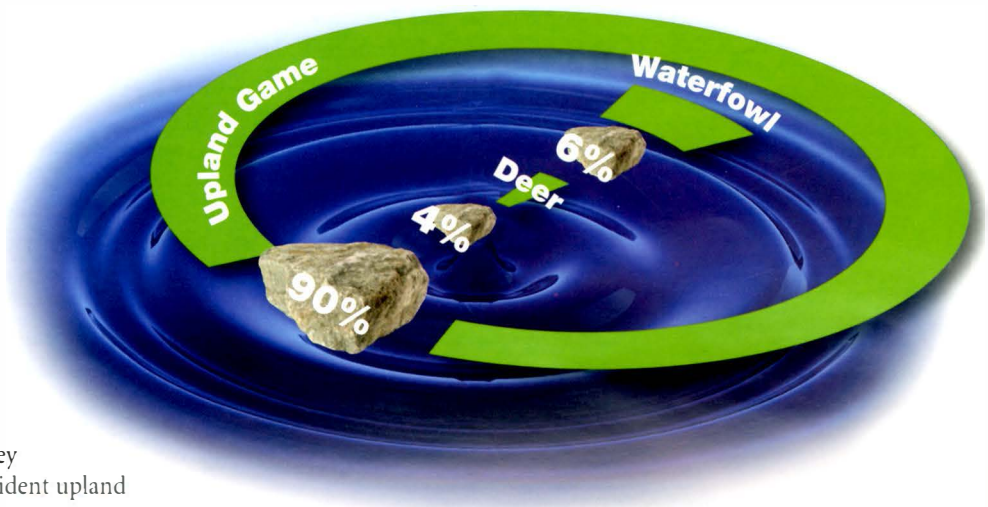
About 56 percent of upland game bird hunters, 23 percent of deer hunters and 21 percent of waterfowl hunters surveyed hunted on CRP land. In 2014, more than 900,000 acres of land in South Dakota was enrolled in CRP.

The 2015 farm bill set stricter guidelines for CRP and Conservation Reserve Enhancement Program and a program cap of 24 million acres nationwide by 2017. That cap is 1.4 million acres less than the acres enrolled in the nation's CRP last year, the lowest since 1988.

If the state had a 50 percent reduction in CRP acres, the survey showed that resident and nonresident upland game bird hunters would make 20 percent fewer hunting trips. The economic impacts from just the reduction in nonresident upland game bird hunters, results in a $12.6 million reduction in economic impacts from those nonresidents, a $6.5 million reduction in total wages and loss of 260 jobs in South Dakota.

"This research shows the beneficial effects on the local economy from hunting that occurs on CRP lands," Loomis said.

Miller hopes this information will be useful to legislators and policymakers. "We're a small state and this is a big impact."



Direct and ripple effect of nonresident hunting

Upland game hunting accounts for more than $109 million, or 90 percent, in direct expenditures from nonresidents, while waterfowl hunting brings in $7 million, or 6 percent, and deer hunting approximately $5.5 million, or 4 percent. The ripple effect of all three types of hunting adds another $72.7 million to the state's economy.

help health-care facilities develop, improve tobacco-free policies

Heidi Mennenga

Jennifer Kerkvliet

care facilities to examine their tobacco policies. The goal is to encourage facilities to develop and/or improve those policies.

"We wanted to work more with health-care facilities on tobacco evidence-based interventions," said Kiley Hump, administrator of the S.D. Department of Health's Office of Chronic Disease Prevention and Health Promotion. "If they ask about tobacco use, do they then give tobacco users advice and referrals to services that will help them quit? If so, how do they do that?"

She pointed out that the South Dakota QuitLine offers a comprehensive range of free tobacco cessation services.

Recognizing strengths, weaknesses

The health-care facilities surveyed included clinics; hospitals; Women, Infants and Children (WIC) and family planning centers; mental health and substance abuse treatment facilities; and cancer centers. Each was asked to provide a tobacco-free or smoke-free policy and answer additional questions regarding tobacco-use assessment and referral procedures for patients, Mennenga explained.

Of the 420 facilities that were invited to participate, 348 provided copies of their tobacco- or smoke-free policy that two research team members evaluated in four general areas utilizing a policy review tool. They looked for a strong, comprehensive policy that was clearly communicated to those who work at or visit the facility. Then they evaluated how complete the tobacco ban was, including what specific products were prohibited, such as chewing tobacco.

In addition, the reviewers considered whether the issue of enforcement was addressed for employees and volunteers, patients and clients, visitors and contractors/vendors—along with disciplinary actions for noncompliance. Lastly, they looked at whether tobacco users were encouraged to quit and then offered guidance on how to do so.

Mennenga shared some summary statistics from the evaluations. Scores ranged from 3 percent to 90 percent. Hospital, clinics and cancer treatment were among the facilities with more comprehensive policies, all scoring above 60 percent. Overall, mental health and substance abuse treatment facilities averaged a 30 percent for comprehensiveness, which mirrors the national trend, she noted.

The researchers found that 77 percent of the facilities had electronic health record systems that prompted them to assess patients' tobacco use. "Among those facilities without electronic health records, only 45 percent had a tobacco cessation section in their intake process," research associate Jennifer Kerkvliet said.

Looking ahead

The researchers have also helped the S.D. Department of Health develop a model tobacco-free policy for health-care institutions that is available at goodandhealthysd.org/healthcare/practice-guidelines.

"We're happy with the two-pronged assessment that addresses, not just buildings and grounds, but also looks at the referral side," Hump said.

Next, the researchers will give each facility feedback on how to improve its policy. The final step will be to conduct interviews at select facilities, representing low, mid and high evaluation scores. "We want to identify barriers and facilitators to developing and implementing tobacco-free policies," Mennenga said.

The researchers hope that their work will result in more health-care facilities developing comprehensive tobacco-free policies and providing tobacco users who want to quit with assistance.

MS

Exercise scientist improves movement, quality of life for MS patients

Bradley Bowser

Kristin Bruns

Groundhog Day 1994 is one Linda Friedrich will never forget. That's the day a neurologist told her, "You have multiple sclerosis and there's nothing we can do." There were no drugs on the market, but Friedrich might be able to get one just coming out—if her name was chosen in a lottery.

The youngest of her three children was only 2 years old, she recalled, and her only vision of MS was a relative who could no longer walk or talk.

Luckily, she got the new drug, Betaseron, within four months of her diagnosis and has experienced only a couple of recurrences which involved numbness from the waist down. "I've been very fortunate," she said, but admits, "my left leg is weaker."

However, when it came to working out in the gym, Friedrich said, "I didn't know what I should be doing." That's why she jumped at the chance to participate in assistant professor Bradley Bowser's research to assess whether strength and flexibility training could help MS patients.

"We're looking at how we can improve function and, at the same time, quality of life," said Bowser, who is the director of the biomechanics laboratory in the Department of Health and Nutritional Sciences. He began working with MS patients in 2007 as part of his doctoral research at the University of Georgia.

Though research has shown that exercise can improve strength and balance for those with Parkinson's disease, Bowser said, "Not a lot has been done in this area with those who have MS, because the disease is not homogenous. Everyone has different symptoms—one person has weakness in one leg, another blurred vision. It depends on where MS attacks the nervous system."

Simple daily living activities, such as handwashing, can become difficult for MS patients due to balance issues, explained Bowser. "Because of problems with balance and instability, MS patients may become less physically active and, thus, more susceptible to illnesses due to a sedentary lifestyle."

How leg weakness affects movement

Bowser and colleagues at the University of Georgia looked at how strength and flexibility training impact muscle function and the mechanics of movement in MS patients. The goal of the study, which was funded through Bowser's doctoral fellowship and the National Multiple Sclerosis Society, was to improve their ability to move from a sitting to a standing position as a means of increasing mobility.

The researchers compared the sit-to-stand motion of 21 individuals with MS in the intervention group with the 12 healthy individuals in the control group. The intervention group was further divided into those with leg weakness and those with normal leg strength.

Bowser reported participants with MS who exhibited leg weakness took longer to move from a sitting to a standing position than those with MS who had leg strength comparable to the control group.

When analyzing their movement, he found those with leg weakness displayed greater trunk flexion and were thrusting their upper bodies forward quickly to create the momentum they needed to compensate for decreased leg extensor strength. The combination of flexion and thrust places additional stress on the lower back, thus increasing the risk of injury, Bowser pointed out.

"Those with leg weakness had to work harder to create similar magnitudes of hip and knee extensor moments," he said.

Measurements showed that MS patients with normal leg strength and the control group had more than 38 percent greater leg extensor strength than those experiencing leg weakness. Less leg strength, in turn, decreased their knee extensor power, he explained. These findings were published last year in Clinical Biomechanics.

In addition, Bowser looked at symmetry and stability during dynamic movement comparing the healthy control group with the MS group before and after the intervention. Preliminary results showed that resistance and flexibility training improved balance and symmetry. However, he noted, "the data set was incomplete."

Looking at exercise, behavioral therapy

A new project begun last summer seeks to determine whether resistance, stability and flexibility training can improve balance and other functional movements for people diagnosed with MS. In addition, Bowser is collaborating with assistant professor Kristin Bruns in counseling and human development to evaluate whether cognitive behavior therapy can provide additional benefits for MS patients.

Cognitive behavioral therapy emphasizes goal-setting, Bruns explained. For example, participants were asked to set specific behavioral goals, which can include exercise. However, behavior therapy also helps clients understand their thoughts and feelings, including coping with depression and anxiety.

The study is funded through grants from the Women and Giving Program at the SDSU Foundation and the SDSU Research and Scholarship Support Fund.

The researchers implemented a 10-week intervention in which all participants did strength, balance and flexibility training for one hour twice a week to strengthen their muscles and improve their balance. However, half the participants also completed a one-hour cognitive behavioral therapy session each week. The training sessions concluded this spring and the results are being analyzed.

Friedrich, who was part of the exercise-only group, said, "I felt good afterward. I might have MS but I can still do this." Her weaker left leg improved but then reached a plateau, she said, "but my right leg and upper body strength kept going."

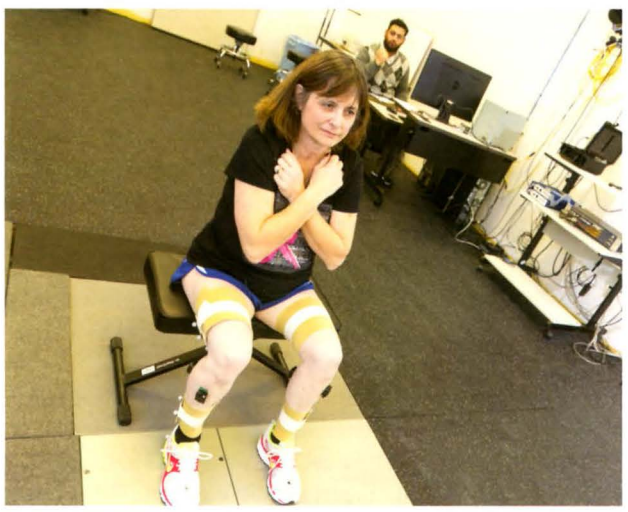
She also noted improvements in balance. Before the training, she pointed out, "if I caught my foot on a rug, I'd be down. Now, I can catch myself and I don't fall."

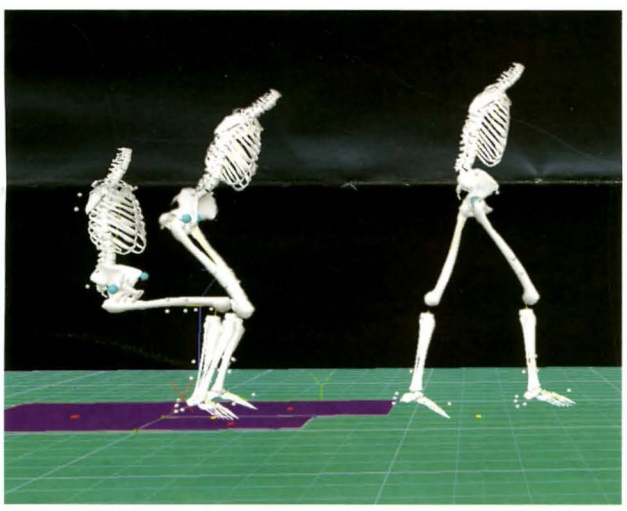
For Friedrich, keeping up with her family is important. "My goal was to walk up Harney Peak when our family went to the Black Hills and I did."

Graduate student Faizan Akram encourages study participant Linda Friedrich as she exercises her weaker left leg. Friedrich, who is from Aurora, was diagnosed with multiple sclerosis in 1994.

"Because of problems with balance and instability, MS patients may become less physically active and, thus, more susceptible to illnesses due to a sedentary lifestyle."

—Bradley Bowser, director of the biomechanics laboratory in the Department of Health and Nutritional Sciences





Above: Data are collected from the two ground reaction force plates Friedrich is standing on while she mimics washing her hands. These data can provide valuable information regarding postural stability and balance during a common activity of daily living where limited stability can be an issue.

Using reflective markers and electromyography sensors attached to Friedrich's legs and torso (see closeup on right), Akram, in the background, tracks the muscle activation, ground reaction forces and joint motion as Friedrich completes what is known as Timed Up-and-Go movement. This involves standing up, walking 3 meters, turning around, walking back to the chair and returning to a seated position.

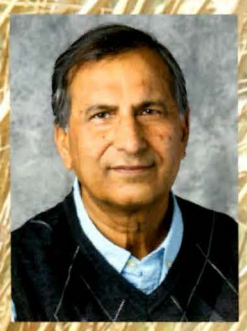
Utilizing the reflective markers placed on Friedrich, the researchers are able to create a skeletal model to examine the mechanics of her Timed Up-and-Go movement.

Plant scientists

defend South Dakota crops against diseases

Protecting the sta arsenal of cultiva predators. It take

Virologist, pathologist guard small grains



Shaukat Ali

Marie Langham

Wheat streak mosaic virus

Tan spot

Bacterial leaf streak

Wheat streak mosaic, tan spot and bacterial leaf streak are among the culprits that virologist Marie Langham and small grains pathologist Shaukat Ali seek to corral. They defend wheat and other small grains against viruses, bacteria and fungi that infest South Dakota fields. The pathologists work with South Dakota State wheat breeders to maintain and develop lines of spring and winter wheat that have resistance to these diseases.

Vanquishing viruses

For Langham, a 25-year veteran, the battle is about timing and resistance.

"With a virus, you have to go with prevention rather than a cure," she said, noting the chief virus affecting South Dakota crops is wheat streak mosaic virus. "It's a fairly mutable virus," Langham said, with Nebraska research showing at least five strains of the wheat streak virus are typically found in each infected field.

Her research is supported by the South Dakota Wheat Commission and U.S. Department of Agriculture Hatch Act funding through the South Dakota Agricultural Experiment Station.

"The natural vector of wheat streak mosaic virus is the wheat curl mite," she explained. "Spring wheat typically germinates and goes through the most susceptible stage before the mites start moving in the spring." Consequently, the mite mainly affects winter wheat.

Wheat streak mosaic requires what Langham calls "a living bridge of hosts" that includes barley, corn, rye, oats and some annual and perennial grasses. Planting nonhost crops and removing volunteer wheat plants can help break that cycle.

But timing can help here, too, Langham explained. Delaying planting until late September—after the mites have migrated—can dramatically decrease the risk of infection.

Working with SDSU's winter wheat breeder, Langham annually evaluates cultivars for resistance to wheat streak. "We want them to be able to withstand aggressive challenges in the fields," she explained, so she inoculates the winter wheat lines with "an isolate that has been very aggressive and at a higher inoculation rate than others might."

When Langham came to SDSU in 1991, a cultivar called Dawn was always at the top for resistance, she recalled. Improved resistance in more winter wheat varieties has pushed Dawn to about one-third to halfway down in the rankings. "We've made improvements that are giving growers more choices," she added.

Defeating fungi, battling bacteria

Ali leads the fight against fungi and bacteria, identifying variation in the pathogens to develop successful disease management strategies, which include fungicide application, and to screen cultivars for resistance. His research is supported by the Minnesota and South Dakota Wheat Commissions and U.S. Department of Agriculture Hatch Act funding through the South Dakota Agricultural Experiment Station.

Ali pointed to tan spot, a fungal leaf disease, as the No. 1 disease affecting durum, spring and winter wheat. "We can lose anywhere from 5 to 53 percent of the crop, depending on the pathogen race and weather conditions."

The pathogen survives on seeds and wheat straw as well as on some grasses, but needs cool, wet weather to produce spores that infect seedlings.

Though scientists have identified eight different races of tan spot circulating in the United States, only five affect South Dakota crops, including Race No. 5 which was observed this year for the first time, according to Ali.

Different genes are associated with resistance to each race, he explained. "If we develop a wheat cultivar resistant to Race 1, it will not have resistance to Race 5 because the gene for susceptibility is different."

Consequently, Ali and doctoral student Sidrat Abdullah are screening commercial cultivars for susceptibility to Race No. 5. In addition, they are using the U.S. Department of Agriculture germplasm bank and the international maize and wheat improvement center CIMMYT to search for sources of resistance that can be used in the SDSU breeding program.

Although fungicides are effective for tan spot, Ali recommends producers vary the type of chemicals they use. "There are already reports in Europe of resistance in this pathogen population to fungicide," said Ali, who also tests for fungicide resistance.

In the last decade, Ali has also seen the re-emergence of bacterial leaf streak. He suspects that an increase in the use of fungicide to combat leaf spot diseases, including tan spot, and to manage wheat scab could open the doors to this bacterial infection. The fungal spores penetrate the leaf and that injury then helps the bacteria get in, Ali explained. The fungicide then suppresses pathogens and subsequently gives the bacteria greater opportunity to spread.

Ali and research manager Richard Gappert have been testing this hypothesis on two wheat cultivars for two years. Those that are inoculated with the fungus that causes tan spot and the bacterium that causes leaf streak and then sprayed with fungicide tend to have more bacterial disease than those infected with bacteria only. "We will do the same experiment this year to make sure," Ali said.

Because bacterial leaf streak is not easy to manage, he noted, "we must rely on breeding." Through CIMMYT, Ali has found several lines of wheat that have resistance to bacterial leaf streak and given those to spring wheat breeder Karl Glover and winter wheat breeder Sunish Sehgal to use in their breeding programs.

By monitoring the diseases that threaten these crops, the pathologists can work with breeders to provide efficient ways of minimizing the damage these pathogens can cause.

Edgar S. McFadden

HOPE WHEAT

Breeding disease resistance part of SDSU legacy

Breeding crops for resistance to disease has a long history at SDSU, beginning in 1913 with Edgar McFadden's effort to combat stem rust, which devastated wheat crops throughout the nation. McFadden successfully incorporated resistance to stem rust by crossing emmer and spring wheat. The resulting Hope wheat variety had resistance to both stem and leaf rust.

ate's food crops requires constant vigilance and an ars that are genetically resistant to the yield-robbing es a team effort.

Pathologists battle foes in soybeans

Fighting sudden death syndrome, an emerging disease that affects soybeans in South Dakota, also means dealing with some familiar foes—Fusarium and soybean cyst nematodes.

Oilseeds pathologist Febina Mathew and SDSU Extension pathologist Emmanuel Byamukama have joined forces to sort out the pathogens and possible interactions of Fusarium with the soybean cyst nematode.

Though soybean producers from neighboring states like Iowa have experienced yield losses due to sudden death syndrome for nearly a decade, the disease did not impact South Dakota producers until 2013. Moist soil conditions and cool weather contributed to an increase in sudden death syndrome in the state.

"The pathogen infects plants through the roots, slowly releasing its toxin over time," Mathew explained. It affects the roots and eventually causes yellowing and then browning of the leaves; however, these symptoms are not apparent until the soybean plants begin to flower.

"It's a relatively new disease," Mathew said. Through presentations at SDSU Extension field days, she and Byamukama have helped producers identify sudden death syndrome. Fungicides are largely ineffective, so farmers must change management practices and select resistant varieties to reduce losses.

"Our research combines field studies with molecular technology to determine which management strategies are truly targeting the pathogens," Mathew explained. "Ultimately, we want farmers to get high yields and maximum economic return on their investments, even under conditions conducive to this disease."

Identifying Fusarium pathogens

A survey of 200 fields in 22 counties showed that approximately 30 fields in 18 counties had signs of sudden death syndrome. However, Mathew noted, only a few of the more infected fields had low to moderate yield losses.

In addition to Fusarium virguliforme that causes sudden death syndome, doctoral student Paul Okello identified nine other Fusarium pathogens in the field samples and determined that Fusarium proliferatum was the most aggressive among the Fusarium species. His findings agreed with those of other researchers in Kansas and Canada.

"We are on the right track with researchers in other states," Mathew said. Mathew is working with Iowa State University soybean breeder Asheesh Singh to screen soybean varieties for resistance to multiple Fusarium pathogens.

Thus far, Okello has identified 13 soybean genotypes with resistance to Fusarium proliferatum that the soybean breeders can use to develop commercial varieties. The research is supported by the South Dakota Soybean Promotion and Research Council and the North Central Soybean Research Program.

Evaluating association with soybean cyst nematode

The researchers have found that sudden death syndrome tends to be worse in fields that have high levels of soybean cyst nematode. Consequently, Okello is looking at possible associations of Fusarium species with soybean cyst nematode.

"This association can be affected by abiotic and biotic factors in the field," Mathew pointed out. Consequently, they are also evaluating whether soil factors, such as potassium, increase the susceptibility of soybean plants to Fusarium, particularly in relationship to the soybean cyst nematode.

To do this, Mathew and Byamukama are working with SDSU Extension entomologist Adam Varenhorst and crop production specialist Jonathan Kleinjan, as well as U. S. Department of Agriculture research agronomist Shannon Osborne. The research is supported by the South Dakota Soybean Promotion and Research Council.

When it comes to the soybean cyst nematode, Byamukama said, "it's purely a management issue because we cannot eradicate it."

The nematode can survive in soil for 10 years without any host. "It's highly prolific. One cyst can have 300 eggs within it and those 300 eggs can reproduce up to three times in a season," he said, noting management is difficult because not all of the eggs hatch at once.

In addition, there are no obvious plant symptoms even when yield losses are already occurring, Byamukama explained. That underscores the importance of periodically testing soil for the cysts, which is free for South Dakota producers, thanks to South Dakota Soybean Research and Promotion Council funding.

So far, resistance has been the most effective way to manage these pests, explained Byamukama, who devotes 25 percent of his time to research. However, "90 percent of resistance comes from one source," he cautioned, "so it may not last for long."

Greenhouse testing has shown 10 to 30 percent reproduction rates on resistant varieties. "Already, we are seeing some pressure," Byamukama said. He counsels producers to rotate crops and vary the resistant soybean cultivars they plant.

In the last three years, Byamukama and graduate student Krishna Acharya have collected soil samples from 28 East River counties to determine which resistance lines are still effective against the soybean cyst nematode.

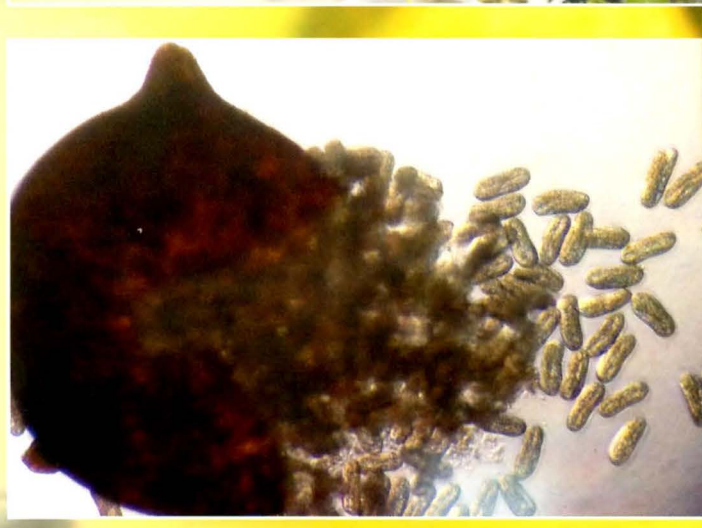
Yearly testing at the Southeast Research Center near Beresford has not yet identified any nematicide seed treatments that have consistently reduced nematode numbers, according to Byamukama. Thus far, resistant soybean cultivars are the best option.

Meanwhile, SDSU's disease-fighting duo will work on understanding the pathogens and factors that affect soybeans' susceptibility to sudden death syndrome.

> "Our research combines field studies with molecular technology to determine which management strategies are truly targeting the pathogens."
>
> —Febina Mathew, oilseeds pathologist

Emmanuel Byamukama

Febina Mathew



Top: Doctoral student Paul Okello compares healthy soybeans with ones infected by Fusarium proliferatum. He identified 10 Fusarium pathogens in plant samples from South Dakota soybean fields and determined Fusarium proliferatum to be the most aggressive.

Soybeans in the second vegetative stage infected by Fusarium proliferatum have underdeveloped root structures in comparison to healthy soybean seedlings.

A soybean cyst is the size of the period at the end of this sentence and can contain up to 300 nematode eggs.

Investigating influenza D virus

earns doctoral student scholarship

Research on a new influenza virus that affects pigs and cattle has helped doctoral student Chithra Sreenivasan earn the Joseph P. Nelson Graduate Scholarship Award. The scholarship, given each year to an outstanding graduate student, recognizes original scientific research and provides $8,500 for tuition and expenses.

She credited her dissertation adviser, professor Feng Li, and her academic adviser, professor Radhey Kaushik, for their support and guidance. "They are the driving forces for my research, and the main reason I received this prestigious scholarship." Both faculty members have joint appointments in the biology and microbiology and veterinary and biomedical sciences departments.

In their recommendation letter, Li and Kaushik describe Sreenivasan as "an exceptional graduate student with outstanding achievement in global, regional and local influenza virology and biomedical research."

Discovering new influenza virus

The new influenza virus, now called influenza D, does not affect humans, Sreenivasan explained. SDSU alumnus Ben Hause '13, now a research assistant professor at Kansas State University, discovered the virus, which he identified and characterized as part of his doctoral work under Li's tutelage.

Li and Kaushik secured a National Institutes of Health grant for nearly $400,000 to continue this work.

Ultimately, the goal is to determine whether the virus can cause problems in humans, Kaushik explained. "If the virus can undergo reassortment in combination with a closely related human influenza virus, it may be able to form a new strain that could pose more of a threat to humans."

Identifying hosts, animal model

To identify exposure to the virus, Sreenivasan tests blood samples for influenza D antibodies. Working with the Minnesota Poultry Testing Lab, she found no evidence of the new influenza strain in poultry. However, she did find antibodies to the virus in sheep and goats from the Midwest through blood samples archived at Washington State University.

Sreenivasan co-authored a paper on those findings that was published in the international journal Veterinary Microbiology last year. In ongoing work, she and her colleagues have also identified antibodies in horses.

Using the bovine influenza D strain, she became the first to prove that the guinea pig could be used as an animal model to study the virus. Though the guinea pigs showed no symptoms, she successfully isolated antigens in tracheal and lung tissues. In addition, her research showed the virus is spread only through direct contact. Those results were published in the Journal of Virology. Sreenivasan was first author of the article.

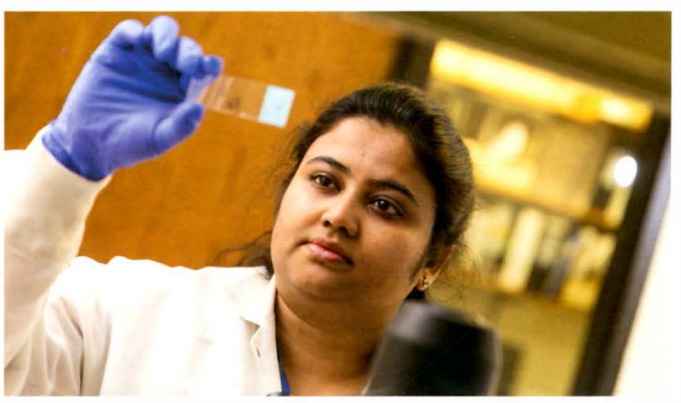
Her current study uses the guinea pig model to compare virulence among bovine and swine influenza D strains and human influenza C. She has just begun analyzing the data.

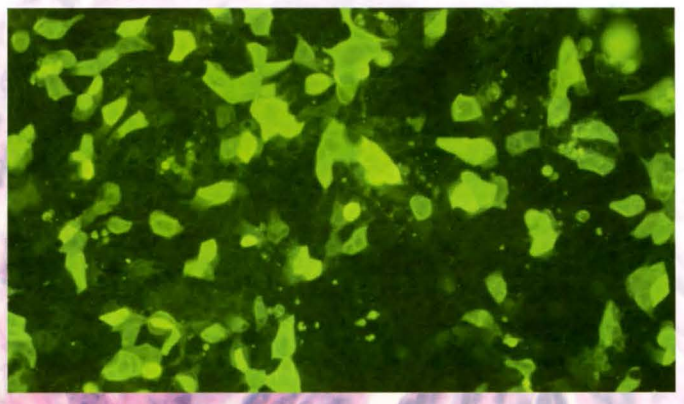
Influenza D has about 50 percent similarity to human influenza C, Sreenivasan explained. "Human C affects mostly children," she said, noting that the most common symptom is a runny nose. "It's not a serious disease. We all have some antibodies because we were infected as children."

In addition, she is developing a way to study the virus in living cells—trachea and lung epithelial cells from swine and cattle. "I isolate the cells and allow them to grow and then infect them to study the genetic and biologic characteristics," she said.

Thus far, she's completed the swine cell cultures and will now begin work on bovine cells. Using the in vitro culturing system, Sreenivasan said, "We will see how the virus attaches and what the receptors are."

As for her scholarship award, Sreenivasan noted, "I will use the money for my research and try to know more about this virus."



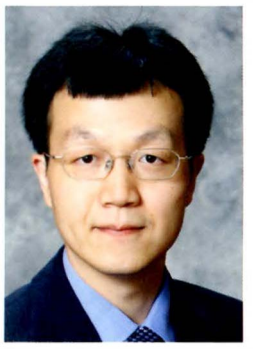


From top: Doctoral student Chithra Sreenivasan examines stained lung tissue sections from guinea pigs to identify the inflammatory changes that occur due to infection with bovine strain of the influenza D virus.

Examination of these lung tissue slides will help her compare the virulence of bovine and swine Influenza D strains and human influenza C in the guinea pig model.

To study the virus in living cells, Sreenivasan uses the indirect immunofluorescence assay. After infection with bovine influenza D virus, she stains the cells—the proportion of green cells indicates the strength of the virus.

Steel shavings trap phosphorous, protecting water quality


Guanghui Hua


Laurent Ahiablame

Routing tile drainage water through an underground bioreactor filled with woodchips has proven effective in capturing nitrates, but a cost-effective method of removing phosphorous is not yet available. That may change—soon.

Researchers from two departments, civil and environmental engineering and agricultural and biosystems engineering, are collaborating to field-test an experimental unit designed to prevent dissolved phosphorous in tile drainage water from polluting lakes and streams.

Assistant professor of civil and environmental engineering Guanghui Hua is using steel byproducts to trap phosphates. He and graduate student Morgan Salo completed lab testing last summer.

The laboratory work, which began in March 2014, was supported by a U.S. Geological Survey grant through the South Dakota Water Resources Institute with matching funds from the East Dakota Water Development District, SDSU Water and Environmental Engineering Research Center and the Department of Civil and Environmental Engineering.

Working with assistant professor Laurent Ahiablame of agricultural and biosystems engineering, the researchers installed a barrel loaded with slightly more than a ton of metal shavings as an add-on to an experimental woodchip bioreactor near Baltic in October 2015. The field-testing is being done through a grant from the South Dakota Soybean Research and Promotion Council.

Experimenting with steel byproducts

Hua and Salo tested four types of steel byproducts to determine their phosphate removal capacity. "These materials are low-cost and readily available for agricultural application," Hua said, noting that they gathered the waste materials from machine shops in the Sioux Falls region.

When the steel shavings rust, the iron oxides that form on their surfaces react chemically, binding with phosphate ions and thus removing the phosphates from the drainage water.

Carbon steel works better than stainless steel, Hua pointed out, noting that the iron oxide on carbon steel surfaces is highly reactive with phosphates. The researchers evaluated the impact of pH, temperature and reaction time on phosphorus adsorption capacity of the steel byproducts.

Based on results from batch testing, the researchers selected a steel mixture containing small and large pieces for subsequent laboratory reactor experiments.

They used column reactors to determine the nitrate and phosphate removal efficiency under continuous flow conditions.

Simulated drainage water was first pumped through a column filled with woodchips and then one filled with steel byproduct. The system contains three times more woodchips than the steel.

The researchers optimized the two-stage bioreactors by varying the nitrate and phosphate concentrations and duration of flow. They tested a range of experimental parameters to simulate field drainage conditions.

During 10 months of continuous operation, the column reactors consistently exhibited 100 percent removal of nitrates and phosphates under typical drainage water conditions, according to Hua.

Protecting lakes, rivers

"This project is a timely one," said Ahiablame, pointing to water quality problems in Iowa, where legal action has been taken because of increasing nitrate levels in the Raccoon River, the source of drinking water for the city of Des Moines.

Jay Gilbertson, manager of the East Dakota Water Development District, agreed. "There's a lot of upside to agricultural drainage, but like anything, there is no free lunch." Though drainage water looks clear and clean, Gilbertson said it often has elevated nitrogen and phosphorous levels.

"We get agronomic benefit from getting water out of the field to optimize production," he said, "but at the same time, we risk increasing the amount of nitrates and phosphates in the runoff."

When farmers install drain tile, they know the steps they are taking to increase production can have negative consequences, noted Ahiablame. "Once we increase tiling, we need to increase water management."

On the upside, tile drainage reduces sediment losses, Ahiablame explained. "Phosphorous has a high affinity to sediment, thus reducing the total phosphorous loading," he said. However, what remains in the drainage water is dissolved phosphorous.

"Phosphates are the leading cause for algae growth in natural water bodies," Hua said. Because dissolved phosphorous is readily available, algae can use it easily. In addition to consuming oxygen, some algae species release toxins into the water.

"That's where this project comes into play," said Ahiablame, noting that he has already received more than 20 calls from farmers about this research. "They are willing to implement this."

Monitoring field trials

In the Baltic bioreactor, drainage water will first flow through the bed of woodchips and then the steel-loaded barrel before entering the last control structure. Water samples are taken before the water enters the woodchips, when it exits the woodchips before flowing through the barrel and as it exits the barrel.

Levels of nitrates, dissolved phosphorous and iron will be monitored. "We want to make sure that we are not creating an additional problem," Ahiablame said, referring to the iron levels in the water.

The researchers hope to remove 80 to 90 percent of the phosphates and nitrates from the tile drain water. Two graduate students, one in civil engineering and one in agricultural and biosystems engineering, will be working on the project.

"We should be able to tell by the summer if this is working or not," Ahiablame said. "The next step will be to determine how we can improve the bioreactor."

Previous studies have shown that an underground bioreactor in the neighborhood of 15 to 20 feet wide and 100 to 120 feet long can handle the nitrate runoff from a 30- to 40-acre field. Similar recommendations will have to be developed concerning the steel shavings needed based on field size.

Ultimately, Ahiablame hopes to develop software that producers can use to design a bioreactor that meets their needs and then helps them determine how much it will cost.

Once an underground bioreactor that removes phosphates as well as nitrates is perfected, producers will have another way of reducing the impact of tiling on water quality.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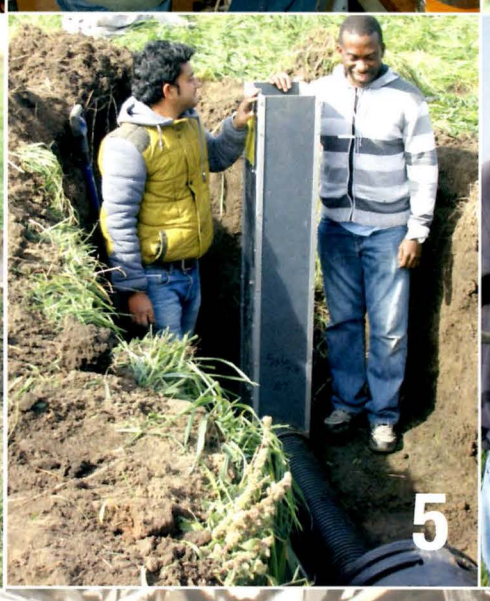
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1. A mixture of small and large carbon steel shavings has been effective at removing dissolved phosphorous from simulated drain tile water in lab tests.

2. During lab testing, graduate student Morgan Salo takes a sample from the upflow reactor to determine nitrate and phosphorous levels. The water runs through the woodchip column on the left and then through the steel-loaded column.

3. Researchers installed this barrel loaded with slightly more than a ton of steel shavings as an add-on to the Baltic woodchip bioreactor.

4. SDSU Extension water resources specialist David Kringen, front, guides the barrel into place with help from adjunct assistant professor Jeppe Kjaersgaard. In the background are: from left, graduate students Bjorn Sellner, Shailendra Singh and Utsav Thapa, assistant professor Laurent Ahiablame and graduate students Alex Boger and Morgan Salo.

5. Thapa and Ahiablame steady the structure that will allow them to take samples after water flows through the steel-loaded barrel.

6. Environment research coordinator Scott Cortus takes a sample before the water enters the woodchip reactor.

INVENTION helps reconnect fish habitat

Roads and highways connect people to one another, much like rivers and streams connect fish populations. However, the culverts that allow water to flow under roadways can become obstacles for small fish, according to associate professor Katie Bertrand of the Department of Natural Resource Management.

"When water flows through the culvert, it falls off and scours out a pool on the downstream side," she explained. The distance from the lip of the culvert to the pool below can be anywhere from a few inches to several feet. When water levels drop during the summer months, moving upstream becomes impossible for small-bodied fish, such as minnows, darters and madtoms.

These observations led to a new invention that will enable small-bodied fish to overcome the challenges of moving upstream through culverts when water levels are low. The patent-pending fish ladder will help minimize the impact that roadways have on fish populations and stream habitat.

"Our researchers discovered a problem and developed an effective means of overcoming it," said assistant vice president William Aylor of the Office of Technology Transfer and Commercialization. He guides researchers through the patenting process that may one day make this invention available to wildlife managers.

Developing, field testing design

Graduate student John Lorenzen built and tested five ladder designs under the guidance of Bertrand and associate professor Brian Graeb. They selected the best design based on lab tests using 20 small-fish species.

"The ladder works for nearly all small-bodied fish," Bertrand said. Only those species that tended to sit on the bottom of the stream pool experienced difficulty using the ladders.

These fish are an important part of the ecosystem, she pointed out. "A grazing minnow, like a Central Stoneroller, common at some of the sites where the ladder was tested, feeds on algae from the rocks and logs on the bottom of the stream." That mitigates the impact of agricultural runoff and algae in the stream.

The researchers then field-tested the ladders in eastern South Dakota and the Black Hills during the summer of 2015 through a $112,086 state wildlife grant from the South Dakota Department of Game, Fish and Parks, with partial funding through the U.S. Fish and Wildlife Service. The fish ladder project was also supported by U.S. Department of Agriculture Hatch funds through the South Dakota Agricultural Experiment Station.

To develop and deploy the ladders, the researchers worked closely with S.D. Game Fish and Parks biologists, Bertrand explained. "This has been a very organic, grassroots effort."

Lorenzen installed fish ladders at 19 sites—nine in eastern South Dakota and 10 in western South Dakota, particularly in the Black Hills.

The ladders were deployed in culverts that ranged from 2 to 12 feet in diameter with the drop-off distance from the culvert lip to the stream ranging from 8 inches to 60 inches. Ladders remained in place for five days, with the researchers collecting fish from a funnel trap just above the ladder each day.

Statewide, 23 species of small fish passed through the ladders. The mean passage rate for ladders in eastern South Dakota was more than 28 fish per day, while West River, it was 1.2 fish per day. Cold-water mountain streams, such as those in the Black Hills, tend to have fewer fish, Lorenzen pointed out.

Preventing habitat fragmentation

The Black Hills National Forest is one of the most densely roaded national forests in the country, according to South Dakota Department of Game, Fish and Parks fisheries biologist Jake Davis. Fragmentation of habitat for native and introduced sport fish is a concern for wildlife managers.

"The ability to pass through culverts would improve fisheries management by increasing the amount of habitat available to them," Davis said.

As adults, these small-bodied fish often move up and down stream, traveling 1 to 2 miles in a day, to find better areas to spawn and feed, Bertrand explained.

"Minnows, for instance, move upstream in the spring during high flow to the headwaters to spawn and then gradually migrate back downstream. Their offspring often stay in the small, shallow headwaters until they are big enough to survive in deeper, faster water."

Davis noted that this invention "could make the complete watershed available to all the fish species in it."

Moving toward commercialization

"Our target client for this product will be state and federal agencies," Bertrand explained, because many road culverts are publicly owned.

The next step for the researchers will be determining how those clients would like to use the ladders, she explained. For instance, should the ladders be deployed only a few months each year or should they be installed once and last for five to 10 years?

Aylor said, "We wish to increase the size of the studies to learn the long-term stability of the ladder and continue to gather data that will help us determine a more effective final product."

Davis envisions state wildlife agencies cooperating with other federal and nongovernment agencies to prioritize locations that would benefit certain species on specific watersheds.

However, because SDSU is not a commercial entity, Aylor said, "We will have to partner with an entity that can produce and deliver the ladders to a customer base or build those abilities with a startup company to support our researchers."

Brian Graeb

Katie Bertrand

Left: The fish ladder that undergraduate student Davis Ahrens and graduate student John Lorenzen positioned on this culvert along Slip Up Creek, northeast of Renner, will allow small-bodied fish to swim upstream to feed and spawn.

Above: Associate professor Brian Graeb, right, hands the fish ladder to Lorenzen. Ahrens, left, and associate professor Katie Bertrand assist with the installation.

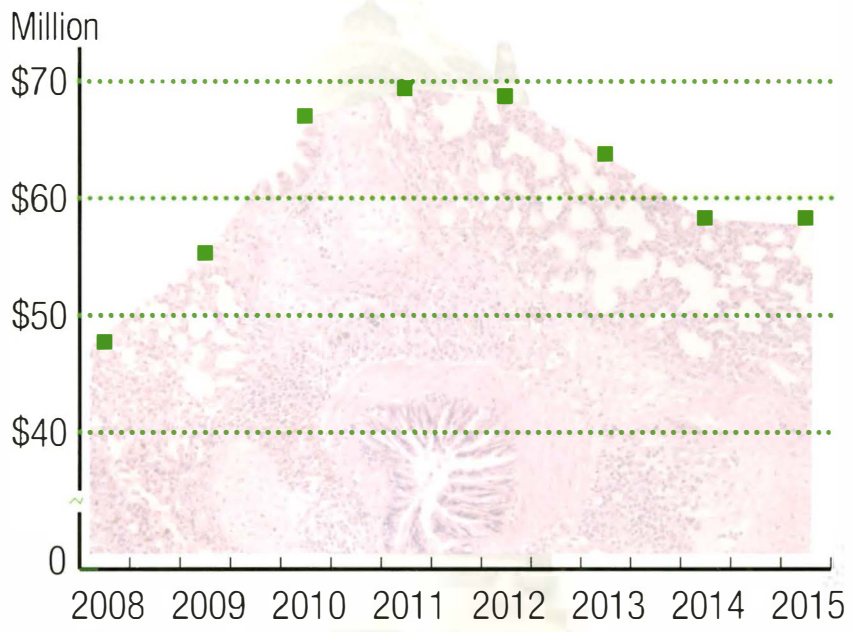
Ahrens installs bolts in the brackets that secure the ladder to the culvert.

Lorenzen sets a funnel trap at the top of the fish ladder to assess how many and what species are using the device to move upstream.

Small-bodied fish, such as minnows, darters and madtoms, can move upstream via the ladder steps.

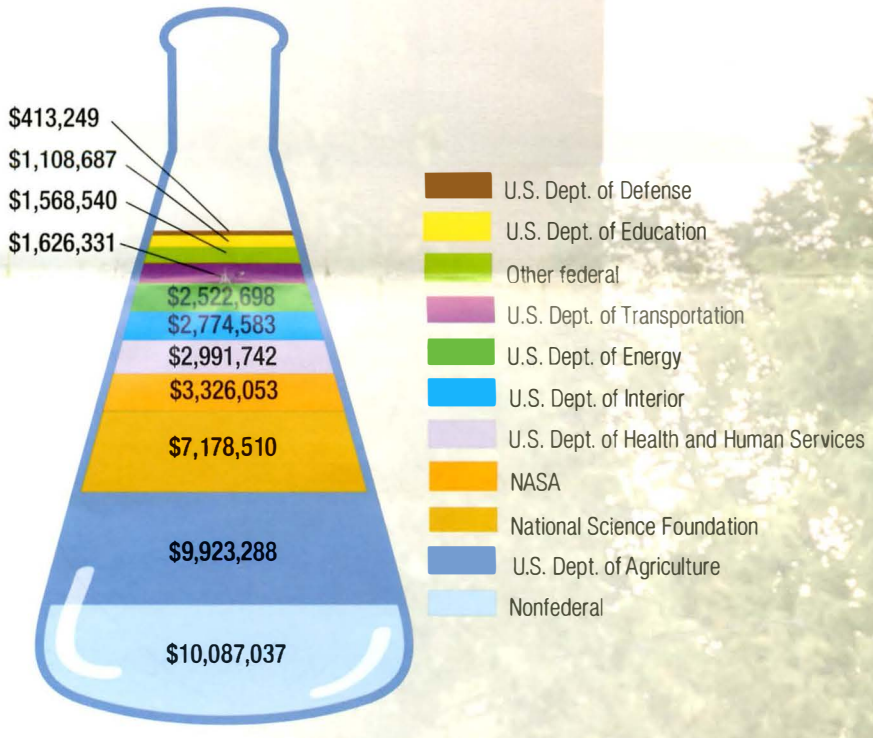
Measuring research investments

Total research expenditures FY 2008-2015*

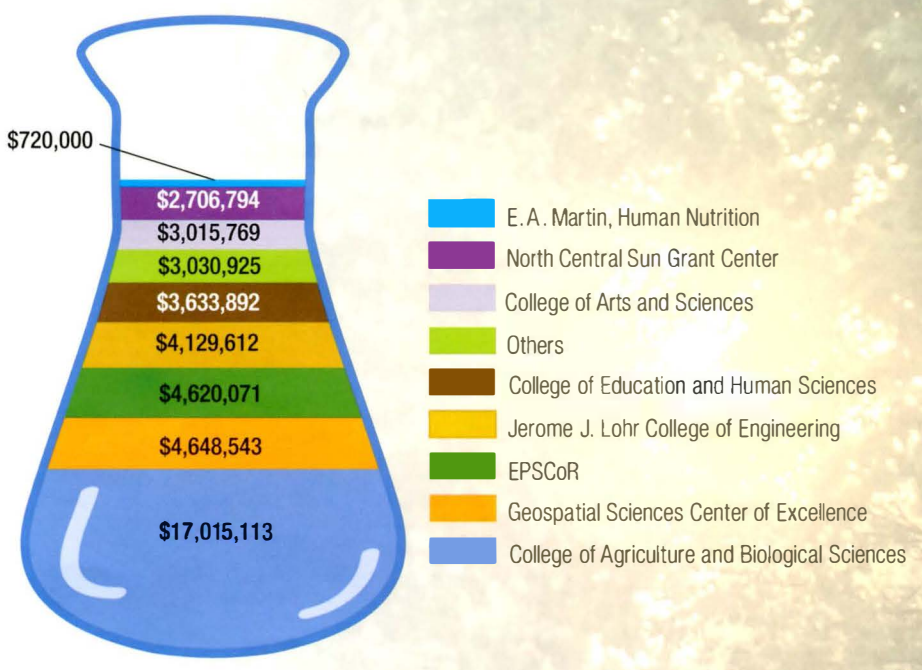


*These figures are the sum of expenditures from grants and contracts, funds appropriated to the South Dakota Agricultural Experiment Station and other institutional funds allocated to research.

Funding sources for FY 2015 expenditures through grants and contracts



FY 2015 expenditures through grants and contracts presented by colleges and research centers



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Commercializing SDSU technologies

Technology transfer

bridges gap to commercialize inventions

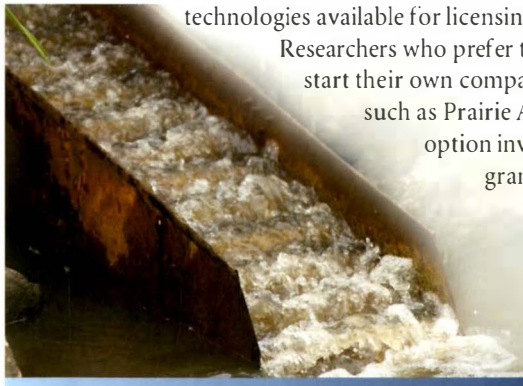

William Aylor

How an SDSU invention becomes commercially available depends on the technology and the researchers who develop it, according to William Aylor, assistant vice president for the Office of Technology Transfer and Commercialization.

The university isn't a commercial entity, he pointed out. "We don't create and sell a product, so we need a partner to help do that." Companies can license SDSU technologies, paying an upfront fee or royalty or a combination of the two, Aylor explained. However, it depends on the technology and its stage of development.

In some cases, such as in the animal health industry, the researchers know which companies may be interested in the technology. If a company can make a vaccine, for instance, and there's a market for it, Aylor noted, "it makes sense to go that route."

When those natural connections don't exist, he said, "we have to go out and identify who the end users are and whether there's a company that can help us out." Aylor and his staff list SDSU technologies available for licensing on the technology transfer Web page.



Researchers who prefer to retain control over their technology can start their own companies, Aylor explained, pointing to examples such as Prairie Aquatech and Tranzderm. However, that option involves recruiting investors and securing grants.

No matter which path the researchers pursue, Aylor helps get the process moving.

More technical information is available through William Aylor, assistant vice president for technology transfer and commercialization—william.aylor@sdstate.edu or (605) 688-4752.

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