

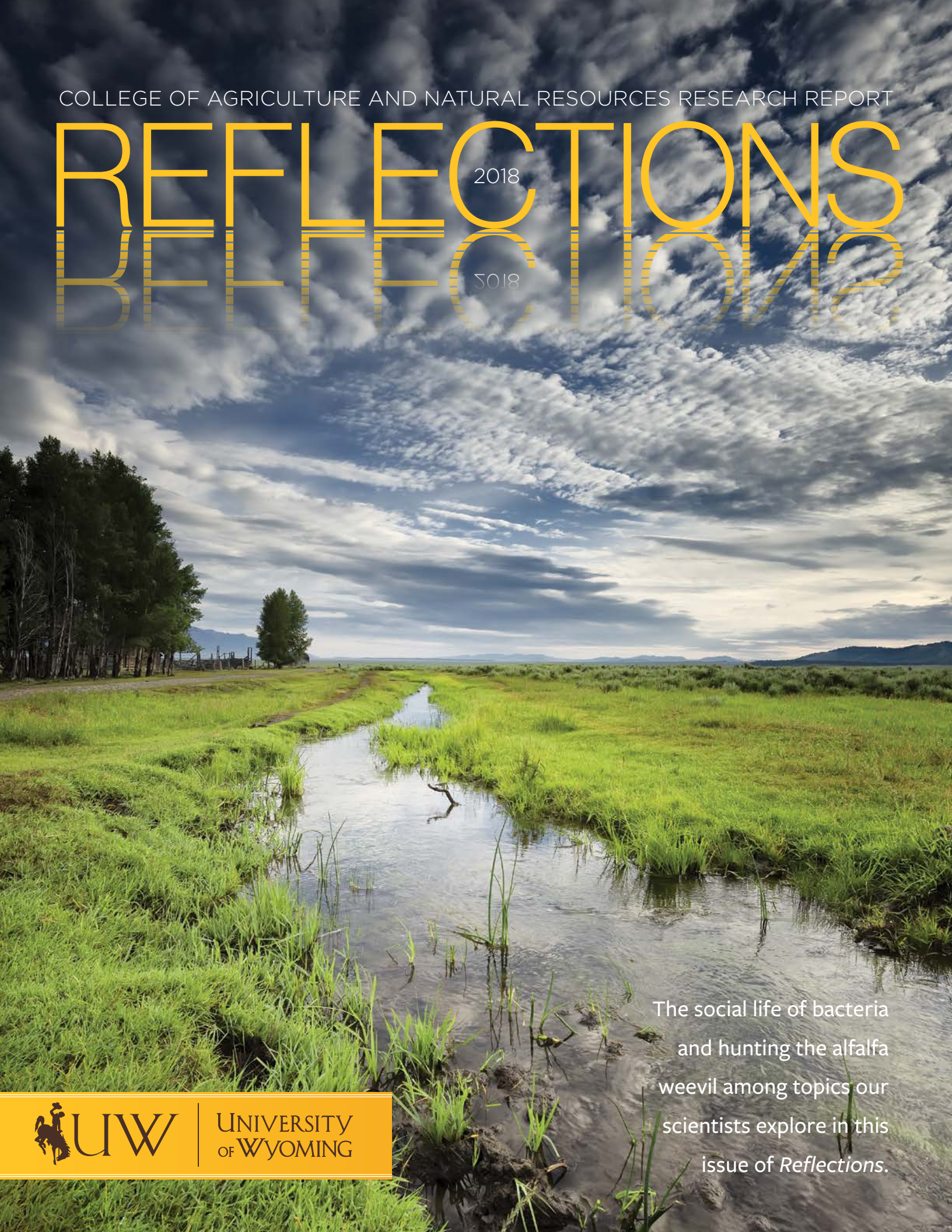
COLLEGE OF AGRICULTURE AND NATURAL RESOURCES RESEARCH REPORT

# REFLECTIONS

2018

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The social life of bacteria and hunting the alfalfa weevil among topics our scientists explore in this issue of *Reflections*.



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# REFLECTIONS

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Welcome to the 2018 edition of *Reflections*, the research magazine of the University of Wyoming's College of Agriculture and Natural Resources.

History continues to be a theme in the college. This year, we say farewell to our long-standing leader. Dean Frank Galey announced his retirement from the University of Wyoming after serving for more than 16 years as the college's dean. However, Frank will not be enjoying the good life of retirement, rather he will be pursuing a new leadership role as executive vice president and provost at Utah State University. The college has enjoyed tremendous support and many successes during his tenure. The college, university, and state are truly indebted to Dean Galey's steadfast commitment to the advancement of agriculture. On behalf of the college and its followers, I congratulate Frank and wish him the very best as the second in command at Utah State.

Associate Dean and Director of UW Extension Glen Whipple also announced his retirement. Glen Whipple is another colleague who has enjoyed a long career as one of the college's leaders, including as head of the Department of Agricultural and Applied Economics and Interim Dean of the College of Agriculture. I thank Glen for his numerous contributions to the college, university, and state. I also wish him all the best in his well-deserved retirement after 33 years of service.

The legend of the two departing college leaders lives on in this edition of *Reflections* – these two were at the helm when each of the contributing faculty authors was hired. I trust readers will agree the research highlighted in the ensuing pages reflects the quality of the faculty members who helped our college leaders successfully address a variety of highly important issues.

I hope you enjoy this issue of *Reflections* and I welcome you to contact me with your comments, suggestions, and questions at [aes@uwyo.edu](mailto:aes@uwyo.edu) or (307) 766-3667.

Best regards,

Bret W. Hess

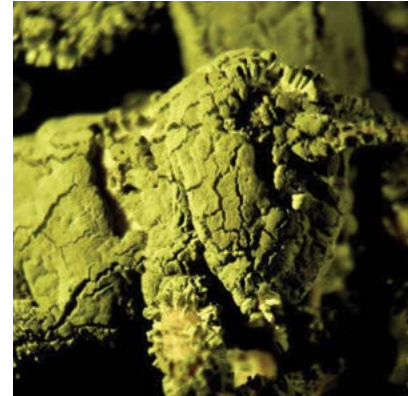
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**YouTube** <https://www.youtube.com/user/UWyoAgExpStation>

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Makenzie Pellissier and Randa Jabbour scout for alfalfa weevil near Lingle.

# The quest to manage alfalfa weevil

**Randa Jabbour**  
*Assistant Professor*  
*Department of Plant Sciences*

Tag along with Assistant Professor Randa Jabbour and research collaborators Makenzie Pellissier and Shiri Noy and technician Zoe Nelson as they hunt the Big Horn Basin for the alfalfa weevil and its nemesis the parasitoid wasp.

June 2, 2015

We pile into the truck in the early morning, the back loaded with coolers, nets, and assorted supplies. Technician Zoe Nelson turns to me, “Did you get a hold of John? Is it so wet we’ll get stuck on that road?” I laugh and respond, “Oh, he says you probably won’t get stuck. You just might slide off the road.” She groans and drives on. We opt to park on the pavement and walk our way in, thus preserving our perfect record of never needing to ask a farmer for a tow (I just jinxed us for 2018).

We are in the Big Horn Basin with one goal: learn how bad the insect pests are in alfalfa fields and how abundant their enemies. If there are enough of these beneficial enemies, then they will kill the insect pests, and producers don’t need to use their money and time to spray pesticides.

Our research focuses on a group called parasitoid wasps: they lay their eggs into pests like alfalfa weevils, those eggs hatch and take over Alien-style, eating out their insides, killing the weevils, then developing into adults that emerge and go on the hunt for more weevils, completing the cycle.

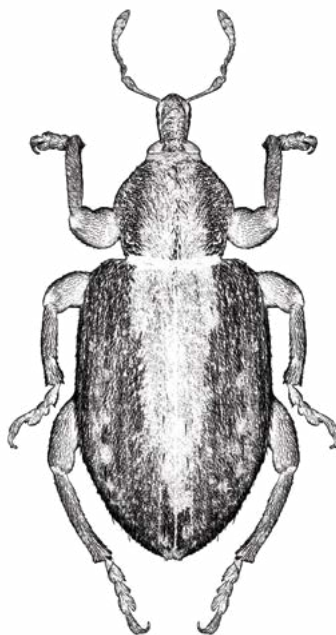
In all our conversations with Wyoming alfalfa producers, alfalfa weevil is the clear winner of the “most problematic pest” award (although seed alfalfa growers surely chime in to nominate the *Lygus* bug). The alfalfa weevil invaded the United States in the early 1900s. In the 1950s and ’80s, in an attempt to control this destructive pest, the United States Department of Agriculture released several species of wasps from the weevil’s home range that only kill alfalfa weevils.



Alfalfa weevil larva

We want to find out: do these beneficial wasps still exist in Wyoming?

As the three of us drive around visiting fields near Lovell and Powell, another team of “weevil warriors\*” are in southeast Wyoming doing the same



thing. At each field, we use our sweep nets to collect insects off the alfalfa, measure plant height, and record which other plants are there and if any of them have flowers.

Sometimes we are happily distracted by wildlife in the fields (my favorite surprise was a huge bullhead snake near Wheatland). Other times, we are racing against thunderstorms, trying not to trip on furrows, and nursing bee stings.

Back at the lab, we set aside some of the weevils from each field to find out how many are parasitized by beneficial enemy wasps. In addition to all the information we collect onsite, we use satellite imagery to quantify what proportion of the land around each field is made up of alfalfa, other crops, or natural habitat. We try to figure out if anything we measured, either at the field or across the landscape, is associated with low weevil numbers and high enemy numbers.





R. Jabbour

Zoe Nelson, left, and Alanna Elder sort field samples in front of a Cody hotel.

“To do science relevant to growers, I carefully listen to farmer perspectives on problems like alfalfa weevil.”

– Randa Jabbour

Weevil adult



### January 8, 2015

How do we know alfalfa weevil is the worst insect pest? Flash back to a few months earlier, when instead of sun-screen and a hat, I opt for a sensible cardigan and audio recorder. I bribe farmers with food in University of Wyoming Extension offices around the state so I can pepper them with questions about how they deal with insects. Why do I do this? My past research showed scientists and farmers think about problems differently. Scientists are more focused on complexity, whereas farmers emphasize management. It’s true that as a scientist I often want to go on and on (and on...) about how complicated plant-insect interactions can be, when a farmer wants to know how to manage the problem. To do science relevant to growers, I carefully listen to farmer perspectives on problems like alfalfa weevil. In these lunch chats, I learn some of the folks in the room have horrible weevil problems every single year and others never ever do.

The discussion turns to possible causes, and the farmers have many ideas. Could it be because Greg’s alfalfa

field is out there by itself, not near any other alfalfa fields? Or that Bob is still haying the alfalfa his uncle seeded before he was born? They have plenty of ideas about what could be driving weevil populations, and here’s the crazy thing - even though scientists have been studying this pest since its arrival 100 years ago, lots of these questions remain unanswered.

### Today

After all the driving, collecting, counting, and calculating, what do we find out?

Let’s start with the alfalfa weevil. We learn low weevil numbers are more common in smaller alfalfa fields than larger ones. Similarly, we find fewer alfalfa weevils in fields surrounded by less alfalfa acreage within a mile or so.

Our farmer focus group was on to something - Greg really might have fewer alfalfa weevils because his field is out there far away from the other ones. Alfalfa weevils only eat alfalfa, so big amounts of it may be more attractive to them. However, we find something else surprising when we look at the surrounding landscape. We find more weevils in fields that have more natural habitat (non-cropland) around them. What could explain this result? We dig into the scientific literature and learn that other scientists suggest alfalfa weevils might overwinter at the base of trees, in the bark.

Perhaps natural habitat that includes trees could actually support higher numbers of weevils. We do not know if this is the case in Wyoming fields and need to do more research to confirm exactly where our weevils overwinter and if neighboring habitat affects that. (Also, to any alfalfa growers reading this, I am not telling you to cut down trees to help your alfalfa weevil problem.). We are constantly surprised by what we learn. The natural world

certainly keeps us on our toes as we try our best to learn its ways.

And what about the beneficial enemy wasps we were trying to find? We find them! In our samples, those wasps kill anywhere from none to 56 percent of weevils, depending on the field. On average, the wasps kill one-quarter of the weevils we collect. This is great news for Wyoming producers.

The disappointing news is we still don't have a clear idea how to increase wasp activity. The number of weevils is the only thing that predicts wasp activity. So, the lower the weevil numbers, the higher percentage of them the wasps are able to kill.

These population dynamics are tricky to parse out from a single point in time. What we do know for sure: if you want to conserve beneficial enemies like these wasps, it is best to limit pesticide application (because some pesticides kill them right along with pests) and give them undisturbed, alternate habitat like flowers in pivot corners or field edges (because adult wasps eat nectar when they are not out killing weevils).

## A Day in the Future

My student Sam Nobres, our undergraduate intern, and I climb into the truck after a long day in the field. "Yes! I am so excited to get this experiment started!" I whoop and turn on some

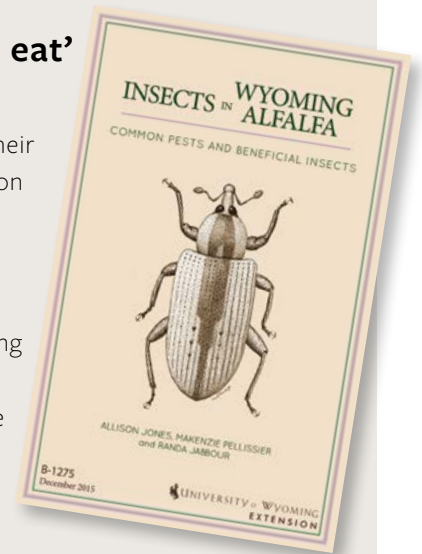


Parasitoid cocoons hatched from alfalfa weevil.

## Alfalfa fields are an 'all you can eat' sign to some insects

MANY insects consider Wyoming alfalfa fields their buffets. See "Insects in Wyoming Alfalfa: Common pests and beneficial insects" at [bit.ly/WYalfapests](http://bit.ly/WYalfapests).

The free to view and download full-color publication is published by University of Wyoming Extension, and whose authors include Randa Jabbour, and divides the state's alfalfa landscape into two parts: the pests and the beneficials, which identifies and describes the parasites, predators, and pollinators in Wyoming fields.



Severe weevil injury to alfalfa

Phil Sloderbeck Kansas State University

festive music. Sam, ever the realist, glances at me out of the corner of her eye, a humoring look on her face. We drive off, leaving the weevils in the dust, to rest up for another day of science surprises, disappointments, and (fingers crossed) victories.

We've only taken our first steps to tackle alfalfa weevils, and we have a lot more work to do.

.....  
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## Acknowledgments

*Thank you to all Wyoming alfalfa producers; our 2015 field crew of Zoe Nelson, Alanna Elder, Preston Hurst, and Jemma Woods; the many other students who worked over the years to count the thousands of insects we caught in those nets; and funding from the Wyoming Agricultural Experiment Station and Western IPM Center.*

## Weevil Warriors



Follow the researchers on <https://www.instagram.com/weevilwarriors/>



# Family, friends, and enemies underfoot

These soil bacteria recognize kin, form social groups, band together to survive

**Daniel Wall**  
*Associate Professor*  
*Department of Molecular Biology*

As humans we learn at an early age who our parents are and that they play a vital role in our well-being. The world around us has many amazing examples of individuals identifying others related to them (Figure 1). Herd animals, flocks of birds, and schools of fish are just

a few other examples where individuals recognize their kin.

The advantage of kin recognition is obvious – related individuals can form social groups to protect and care for the young and sick, provide defensive strategies against predation, and generally allows groups to perform tasks beyond the capabilities of an individual. Kin recognition creates strong societies that improve the fitness of group members to succeed and reproduce.

Kin recognition is not limited to animals; microbes also recognize kin and form social groups.

## A Molecular Biologist's View

Molecular biologists seek to explain how living systems work by reducing life to its simplest components. They discover the molecules of life and define their functions. Once the players (proteins) and the genes that encode them are identified, their functions are studied and models are built to explain how biological processes work. This approach is extremely powerful and has led, for example, to the discovery of the genetic basis of many human diseases and later, the therapeutic molecules to cure diseases.

Although there are innumerable success stories, molecular biologists cannot answer every biological question. In some cases, processes are just too complicated to be reduced to molecular terms.



**Figure 1.** Examples of animal species that form kin groups. The lower right panel shows myxobacteria also form kin groups as seen under an electron microscope.





**Figure 2.** Photograph of a myxobacterial colony (left) consuming an *Escherichia coli* colony (right). The myxobacteria ripples (black arrow) are the results of synchronized movements among thousands of cells. Cells erect fruiting bodies where nutrients are absent (white arrow). Click link to watch movie.

Case in point, kin recognition in animals is complex, involving the five senses, memory, and cognition. Reducing all of these processes to molecular steps is currently beyond our capacity.

In contrast, bacteria are relatively simple life forms that also use kin recognition. Molecular microbiologists, such as myself, have the experimental tools to understand how this fascinating process works at the molecular level.

### **Myxobacteria – a highly social group of microbes**

We study a group of microbes called myxobacteria. These bacteria are known for their spectacular social behaviors including gliding motility (movement) and their ability to hunt as “microbial wolf packs” (Figure 2). Their ability to aggregate about a million cells and build multicellular structures called fruiting

bodies is their most notable behavior (Figure 3, page 10). Fruiting bodies form in response to starvation wherein cells differentiate into spores, which are dormant cells that resist environmental stresses and can survive for years without nutrients.

Upon dispersion to new locations, spores germinate, providing nutrients are present, and cell growth resumes. For myxobacteria, multicellular aggregation is a challenging strategy because their natural habitat, soil, contains a dazzlingly diverse array of species and individuals, presenting the problem of how to identify kin to build a multicellular organism.

### **From Jelly Beans to Fruiting Bodies**

We discovered myxobacteria express a receptor, called TraA, on their

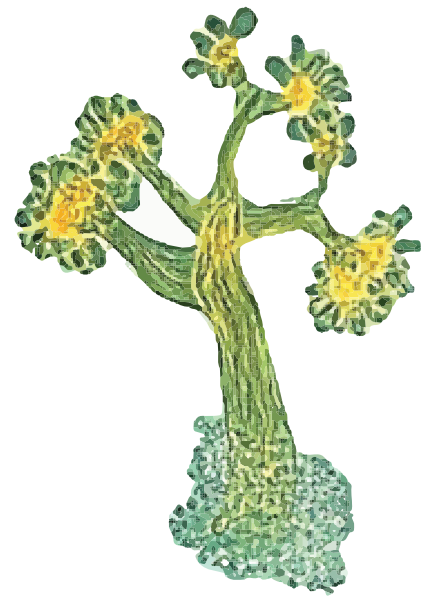
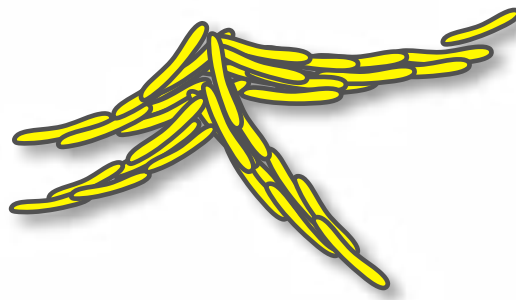
cell surface that allows them to recognize or “see” others who bear identical receptors to themselves. Specificity in recognition occurs because the receptors are highly variable in natural myxobacteria populations.

As an analogy, imagine a pile of jellybeans consisting of many different colors, where each jelly bean represents a myxobacteria cell and each color represents a TraA receptor that confers unique specificity (Figure 4). In this analogy, only jellybeans of the same color recognize each other. These related or identical cells stick together and alter their gliding motility such that they form distinct social (color) groups.

These groups hunt together in microbial wolf packs or, if no food is available, they aggregate and build fruiting bodies.

**Figure 3.** Schematic for how myxobacteria aggregate and form a multicellular fruiting body. Opposite page, a pile of jelly beans represents the enormous degree of microbial diversity found in soil. TraA receptors help myxobacteria form distinct social groups depicted by red, yellow, and green jelly bean piles. In response to starvation, thousands of individual cells aggregate and build a multicellular fruiting body.

## Starvation and development



As molecular biologists or genetic engineers, we seek to reengineer biological systems to do something new or unique. The ability to reengineer cells has practical applications, like the production of therapeutic drugs, as well as the demonstration of mastery over how a biological process works. In this regard, we demonstrated how kin recognition works with TraA.

First, we showed recognition could be reprogrammed so a “green cell” could be changed to recognize a “red cell” by simply swapping which type of TraA receptor the cell contained. We did this by genetically removing the TraA receptor from a “green cell” and substituting it with a receptor from a red cell. Such genetically engineered cells now recognize red cells as kin instead of their green parental cells.

Secondly, at a more refined sub-molecular scale, we also reengineered the TraA receptor itself by changing the protein sequence so that not only was the specificity of recognition changed, but we also created completely novel recognition groups. We did this by creating chimeric receptors (the fusion of different receptor pieces together) or by simply changing single amino acid residues in the receptor sequence.

### Rejuvenating Siblings

Individuals within a society eliciting preferential treatment to others related to them is a hallmark of kin recognition. For example, in mammals, parents share resources with their offspring to facilitate their development, which helps ensure survival of offspring that contain their parents’ genetic material. Similarly, myxobacteria that contain identical TraA receptors will exchange or share their cellular content with their siblings.

The exchange of cellular cargo includes proteins and lipids but not DNA. One consequence of sharing is that healthy cells can repair damaged or sick siblings. Rejuvenation occurs because cells that contain damaged or missing components are replenished with vital cellular factors from their healthy siblings.

In the laboratory, we demonstrated that cells defective in motility, because an essential motility factor was broken or missing, regained motility by contacting siblings that “donated” the missing protein function. Similarly, cells that were dying because their membranes were damaged were rejuvenated by TraA recognition and membrane exchange from healthy siblings.

Interestingly, healthy cells also

benefited from exchange because a larger and stronger society was created, allowing a population to perform multicellular tasks, such as development, which requires a threshold number of cells.

### Visualization of Kin Recognition at the Molecular Level

Cells are made up of molecules that serve as the building blocks of life. To understand life, we must explain how its molecules function. To observe how kin recognition works, we engineered TraA to contain a red fluorescent tag. Similarly, to visualize cellular exchange, we placed a green fluorescent tag on cellular cargo. By use of live cell imaging, we then visualized the process of TraA recognition and cargo exchange under a microscope.

As shown in Figure 5, when a TraA-red labeled cell makes contact with an exchange partner that contains green cargo, the TraA receptors coalesce between cells to form foci on the cell surface, indicating homotypic recognition occurred with another cell that bears an identical receptor. Shortly after the TraA foci form, the green cargo is transferred into the red cell. Although not depicted in this figure, the original green cell also forms TraA foci



## Kin recognition

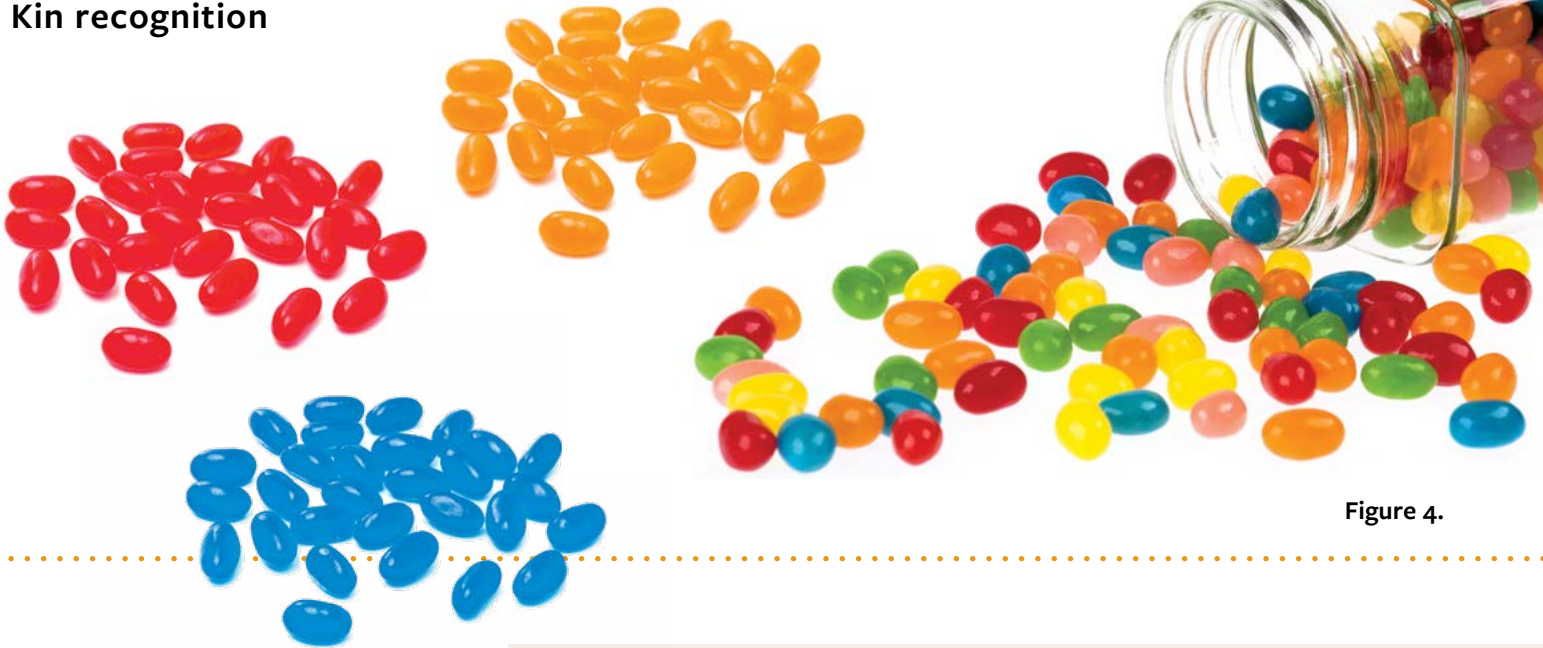


Figure 4.

and reciprocally receives cargo from the red cell.

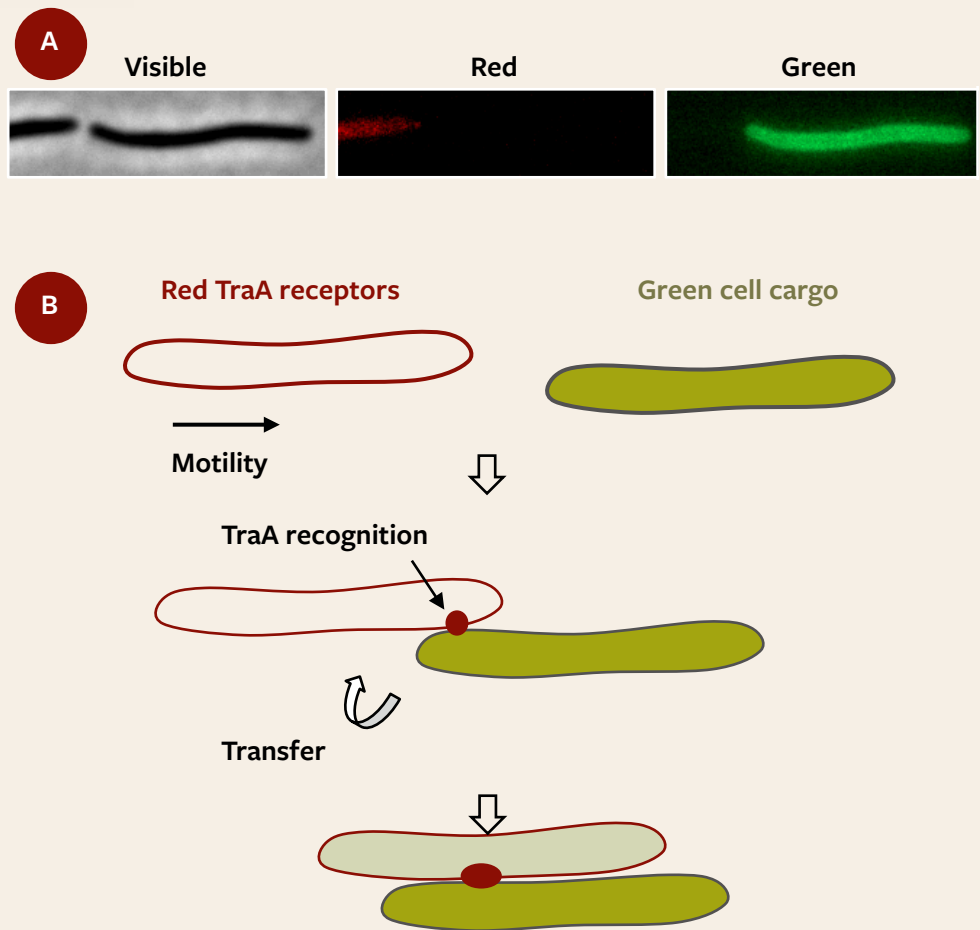
### Future Direction

Going forward we hope to program myxobacteria to perform interesting and beneficial social behaviors. One such behavior is microbial predation where myxobacteria hold promise to serve as biocontrol agents that protect crops from soil derived pathogens. Since predation is a cooperative behavior among myxobacteria, it has the potential to be engineered and optimized.

Myxobacteria are also important to drug companies as producers of natural products clinically used to treat cancer or hold promise as new and novel antibiotic drugs. Researchers have found that some natural products are linked to social interactions that may be amenable to manipulation.

Beyond myxobacteria, we think our discoveries will provide insights into how other kin recognition systems work and how they might be reprogrammed for human gain.

**To contact:** Wall can be reached at (307) 766-3542 or at [dwall2@uwyo.edu](mailto:dwall2@uwyo.edu). His laboratory information is at [bit.ly/juwmolecularwall](http://bit.ly/juwmolecularwall).



**Figure 5.** TraA mediated kin recognition and cargo exchange in myxobacteria. A) Microscopy images of two interacting cells. Left cell contains a TraA receptor labeled with a red fluorescent marker. Right cell has the same receptor unlabeled but expressing green labeled cargo. The pictures show the same image under three different light channels. B) Cartoon illustrating what the movie in A shows.



# WHAT DID YOU SAY?

## Bacteria could grow your future shirt?

**Jennifer Harmon**  
Assistant Professor

**Logan Fairbourn**

**Natalie Thibault**  
Undergraduate students,  
Department of Family and Consumer  
Sciences

Rising consumer demand for affordable, fashionable clothing is fueling unsustainable practices in the textile industry.

This cycle has resulted in unmanageable waste, drawing the industry and academia toward developing alternatives to traditional fibers. Bacterial cellulose, a web of micro-cellulose fibers produced by the small, acetic-acid bacterium *Gluconoacetobacter xylinus*, is one such alternative.

Our research group set out to measure bacterial cellulose performance as a textile. We investigated the material's properties and cellulose yield to understand the potential for apparel and textile applications.

Currently, costly media is needed to grow bacterial cellulose, so we also tested four low-cost nutrient media

against two standard media (Hestrin-Schramm) to optimize production of an affordable end-use material:

1. High fructose corn syrup,
2. High fructose corn syrup and mannitol,
3. Molasses,
4. Molasses and mannitol,
5. Hestrin-Schramm, and
6. Hestrin-Schramm and mannitol.

Half of each group was dried at room temperature and humidity while the other half was dried using an industrial freeze-dryer set to -35 to -42 degrees Fahrenheit to determine the impact of drying processes on textile performance.

### What We Did

- Tensile strength was evaluated using an instrument that stresses the cellulose and calculates the maximum stress and strain.



- Abrasion testing was performed using an instrument that abraded a circular swatch under stress to determine the number of rotations the material could withstand before a tear or hole appeared.
- To determine cellulose production and compare yields across the various media, average thickness (mm) was determined along with final dry weight.

### What We Found

The molasses-mannitol medium produced the greatest amount of cellulose at nearly 8 grams per liter for freeze-dried treatments and 5 grams per liter for air-dried treatments (Figure 1).

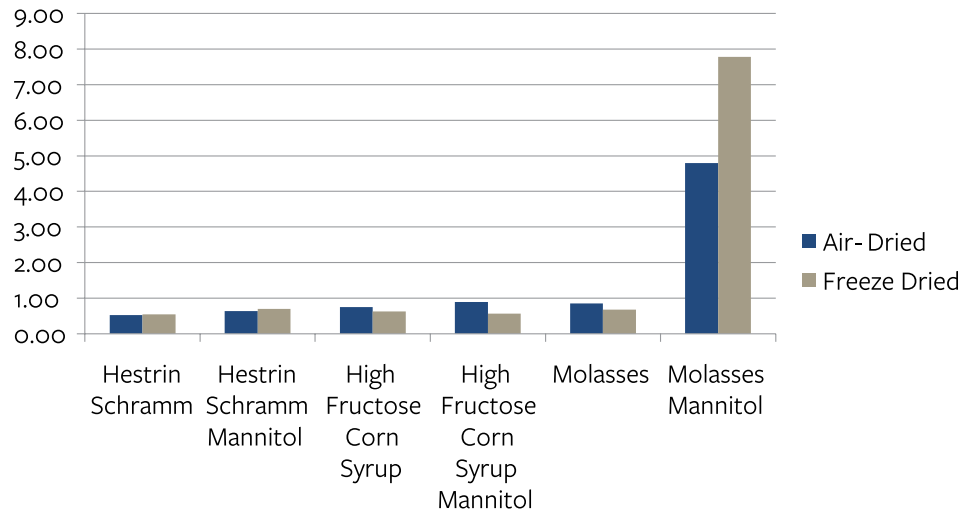
The molasses-mannitol cellulose mats were also the thickest (Figure 2) averaging 0.53 mm for freeze-dried samples and 0.28 mm for air-dried samples. These thicker mats also produced a wide degree of variation between the 15 points of measure.

The molasses-mannitol medium also produced bacterial cellulose with the greatest tensile strength at 38.26 Newtons (N) for freeze-dried samples and 14.17 N for air-dried varieties (a little less than 5 N equals about 1 pound of force) (Figure 3).

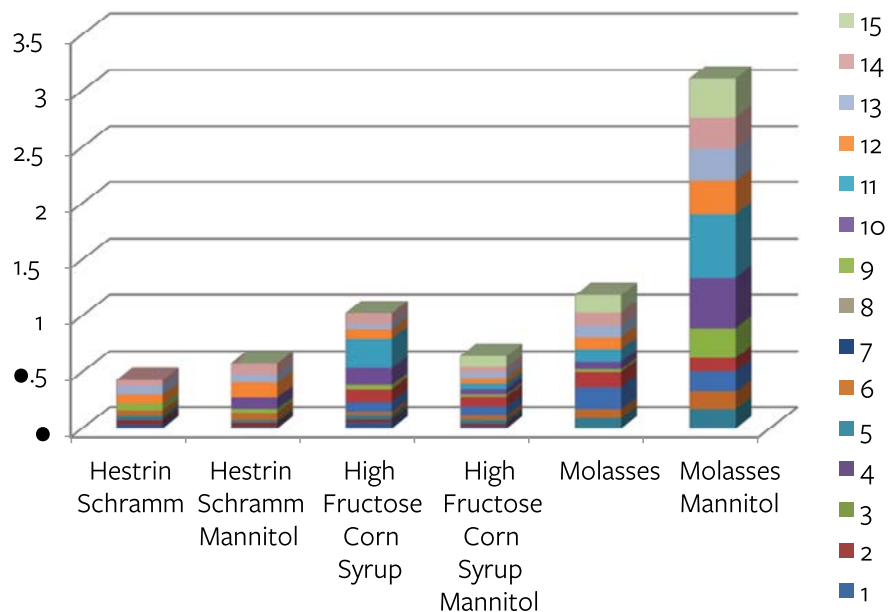
Impressively, the molasses-mannitol bacterial cellulose showed the best resistance to abrasion, illustrated by samples capable of withstanding up to 5,000 abrasion cycles for air-dried and freeze-dried varieties without producing a hole or a tear (Figure 4). This resistance to wear and tear could make this material appropriate for high stress end uses, such as upholstery or straps.

Our project showed that using molasses-mannitol media could increase

**Figure 1. Grams of cellulose for final product by media type**



**Figure 2. Points of measure variation mm air-dried**



### Why bacterial cellulose?

Bacterial cellulose fibers are biodegradable and lack lignin/hemicellulose polymers, resulting in a textile similar to vegetable leather. Hydrogen bonds between the micro-cellulose fibrils impart great strength to bacterial cellulose. The result is a strong, eco-friendly material with the potential to replace some traditional cellulosic textile materials.



Natalie Thibault, left, and Logan Fairbourn, right, perform abrasion tests on sustainable fabric with faculty mentor Jennifer Harmon.

production of bacterial cellulose by up to 800 percent over standard nutritional media.

The next step in developing bacterial cellulose as a marketable textile alternative is to see if we can produce the material as a woven fabric. A major limiting factor is that bacterial cellulose is typically a nonwoven, fiber-web material as opposed to a yard-based, woven textile. Most consumer textiles are woven, which imparts better hand and breathability. Harmon's team aims to investigate this objective using 3D printing and fiber molding techniques.

*This research was made possible by a University of Wyoming Faculty Grant-In-Aid award.*

**To contact: Harmon** can be reached at (307) 766-5669 or at [jharmo14@uwyo.edu](mailto:jharmo14@uwyo.edu).

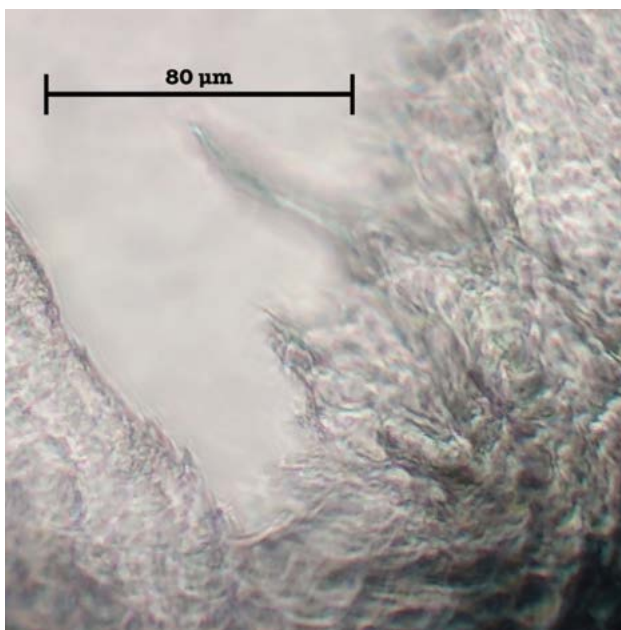
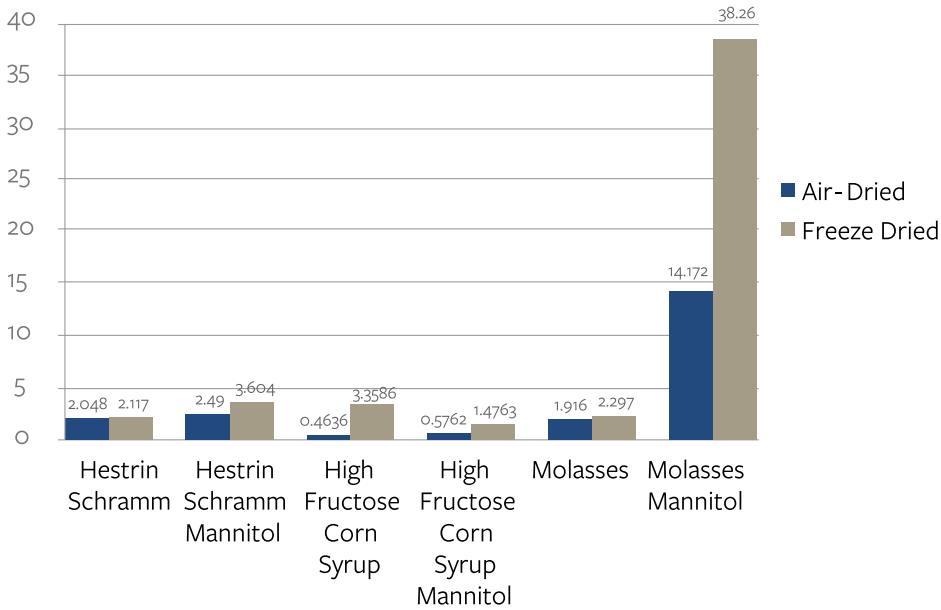


Image at 1,000x magnification of air-dried bacterial cellulose in oil immersion microscopy.

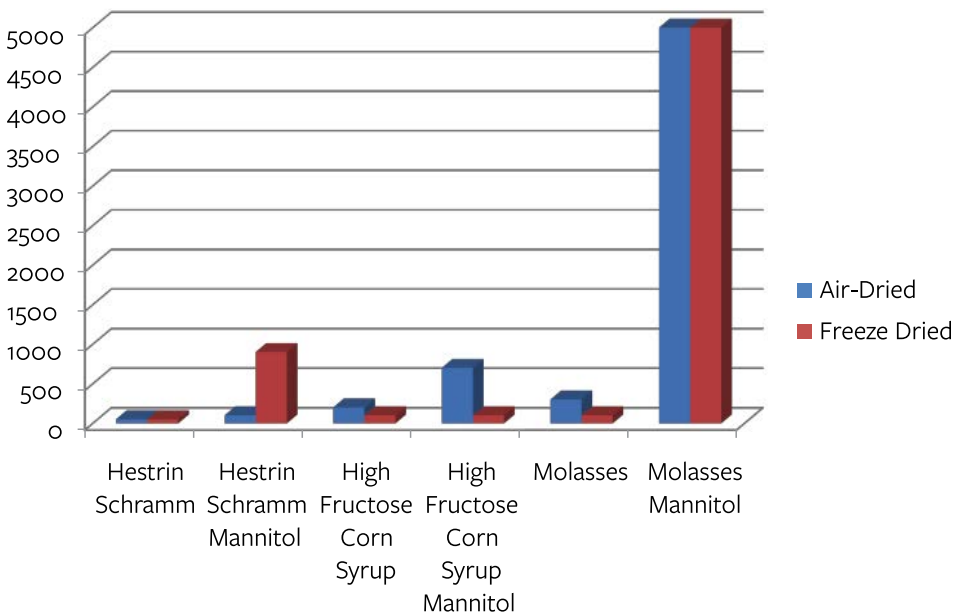


**Figure 3. Strength: N before breaking**



Air-dried

**Figure 4. Abrasion resistance: Rotations before damage or end of test**



Freeze-dried

Harmon's research group will investigate organic dyes to produce customizable bacterial cellulose. Research team members hope to develop a standard operating procedure for producing and aesthetically modifying this cellulose. This operating protocol could lend itself to large-scale bacterial cellulose production as a textile alternative in areas like Wyoming that lack longer growing seasons and arable land conducive to producing traditional textile materials, like cotton.

# A Molecular Bungee

## Manipulating ‘rubber band’ protein could provide path to novel heart failure treatment

**Wei Guo**

*Assistant Professor  
Department of Animal Science*



<http://bit.ly/molecularbungee>

Land diving or vine jumping is a ritual carried out by certain natives of Vanuatu. The land diving was the precursor to bungee jumping – an extreme sport not for the faint of heart.

Vine jumpers commonly are injured because vines are much less flexible than bungee cords. When people jump and free-fall from a great height, the bungee cord, like a rubber band or spring, is stretched from a relaxed position to generate passive force to absorb the energy from the descent. The elastic bungee cord saves the jumper a lot of internal damage – and psychological trauma.

Amazingly, nature also employs elasticity to dampen biological forces or generate passive stiffness at the molecular level, such as muscle stretching during exercise. The molecular bungee cord

that serves this purpose in the human or animal muscle fiber is the protein titin, a giant protein that is the largest

identified so far in vertebrate animals.

Titin functions as a molecular rubber band to

provide stiffness to protect muscle fibers from damage due to overstretching. Titin is the third most abundant protein in muscle tissue after myosin and actin. These three proteins work together in a basic unit of muscle tissue called a sarcomere (Figure 1), which helps control muscle contraction.

Sarcomeres have stacked filaments that slide past each other when a muscle contracts or relaxes: myosin is a thick filament, actin is a thin filament, and titin binds to both anchoring them into position.

The ratio of small and large form titin is important to understanding the mechanisms of heart failure because the small form is stiffer and the large form is more compliant or less stiff (Figure 2). My laboratory is working to determine how the proportions of these two forms of titin are controlled, with the ultimate goal of developing a novel therapy for the treatment of heart failure.

### Stiff Hearts and Failure

Heart disease is a serious health problem in America – it’s the leading cause of death in the U.S. for both men and women, resulting in 1 in every 4 deaths annually. In Wyoming, heart disease is also the number one killer with 1,035 deaths in 2014; cancer was second with 922 (Figure 3, page 18).

One type of heart disease is heart failure. Nearly 6 million Americans



# Cord

are living with heart failure, and 1 in 5 Americans are at risk of developing it.

Heart failure does not mean the heart no longer works. Heart failure means the heart is unable to pump sufficient blood to meet the body's need for oxygen. In other words, the heart can't keep up with its workload.

There are two types of heart failure.

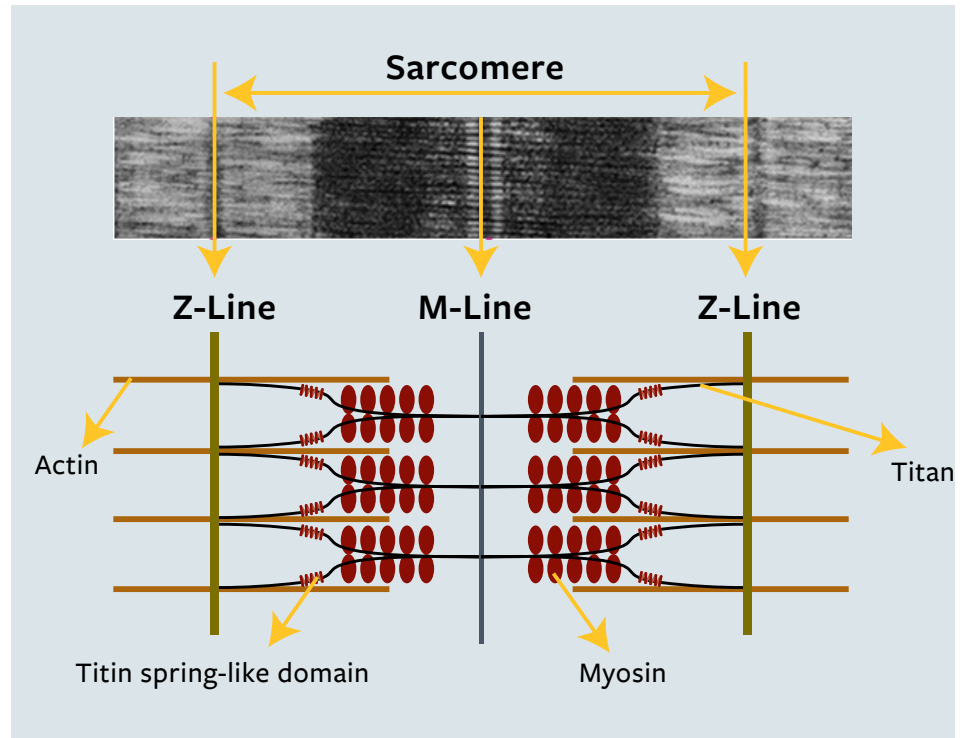
**Systolic** failure or dysfunction is when the left ventricle loses its ability to contract properly, and the heart can't pump with enough force to push sufficient blood into circulation.

**Diastolic** failure or dysfunction occurs when the left ventricle is stiffer and loses its ability to relax normally, and the heart can't properly fill with blood during the resting time between each beat.

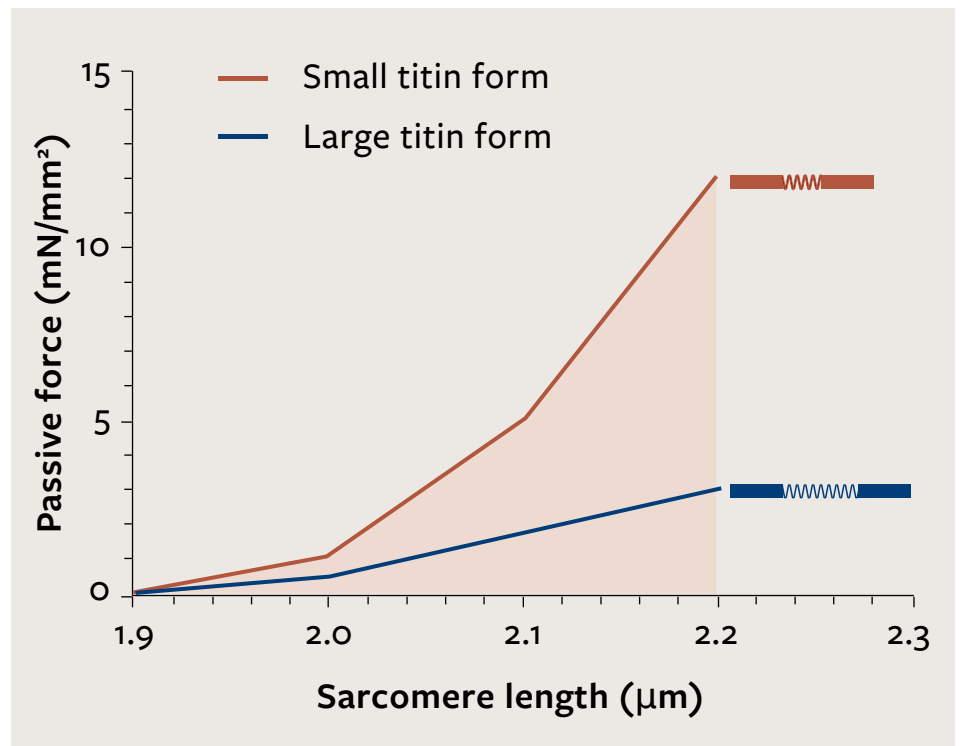
Both complications contribute to heart failure in about an equal number of patients. Treatments differ for the two types because of the distinct properties of each.

There are no treatment options to reduce heart wall stiffness for diastolic failure. Titin, due to its elastic property, has been considered as a promising therapeutic target to treat diastolic failure. Ours and other research laboratories have found that failing hearts with diastolic dysfunction express higher levels of smaller titin form, while failing hearts with systolic dysfunction express higher levels of larger titin form (Figure 4, page 19).

Correcting the aberrant ratio of these two forms could be a potential therapeutic strategy for the stiff heart with diastolic dysfunction. The challenge

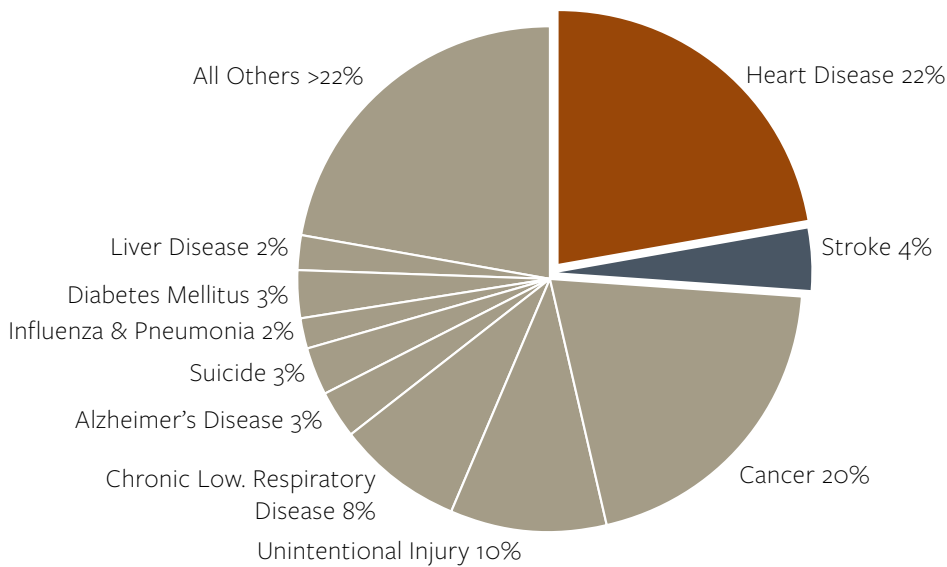


**Figure 1.** Upon muscle contraction, actin slides in and out along the myosin in the middle of the sarcomere, and titin binds to both actin and myosin stabilizing them in position.



**Figure 2.** Titin exists in two forms in the heart. The smaller form produces greater passive stiffness, like taking a short rubber band and stretching it; more force is needed to hold it the farther it's extended.

## LEADING CAUSES OF DEATH IN WYOMING 2015



### HEART DISEASE

is the number

1

killer in Wyoming

1,030

people in Wyoming  
died of heart disease  
in 2015

### STROKE

is the number

5

killer in Wyoming

198

people in Wyoming  
died of stroke  
in 2015

is determining the factors that control their expression.

### Factors Controlling for Two-titin Form Switching

Researchers originally thought one gene should encode for one protein, but later studies indicated this was not true. The Human Genome Project identified approximately 25,000 protein-coding genes, but we know the human genome can synthesize many more than the 25,000 proteins expected if one gene encoded only one protein. The reason is a biological process called “alternative splicing” whereby a single gene can code for multiple proteins.

In this process, one class of proteins called splicing factors plays a critical role.

Our research revealed a splicing factor called RNA binding motif protein 20 (RBM20) is responsible for the expression of two titin forms. In the **presence** of RBM20, the major form expressed in the heart is the smaller one. In the **absence** of RBM20, the larger form is exclusively expressed (Figure 5A).

Interestingly, we found that even when RBM20 is present in the

## HEART DISEASE AND STROKE RISK FACTORS IN WYOMING

	Wyoming	U.S.
Adults who are current smokers	19.1%	17.5%
Adults who participate in 150+ min. of aerobic physical activity per week	54.4%	51%
Adults who are overweight or obese	65.4%	65.3%
Adults who have been told they have had a heart attack	4.7%	4.2%
Adults who have been told they have had a stroke	2.8%	2.9%
Adults who have been told they have angina or coronary heart disease	3.4%	3.9%
Population of adults (18-64) who have some kind of health care coverage	86%	91%
High school students who are obese	11%	13.9%

Figure 3. Wyoming State Fact Sheet in 2015 from the American Heart Association



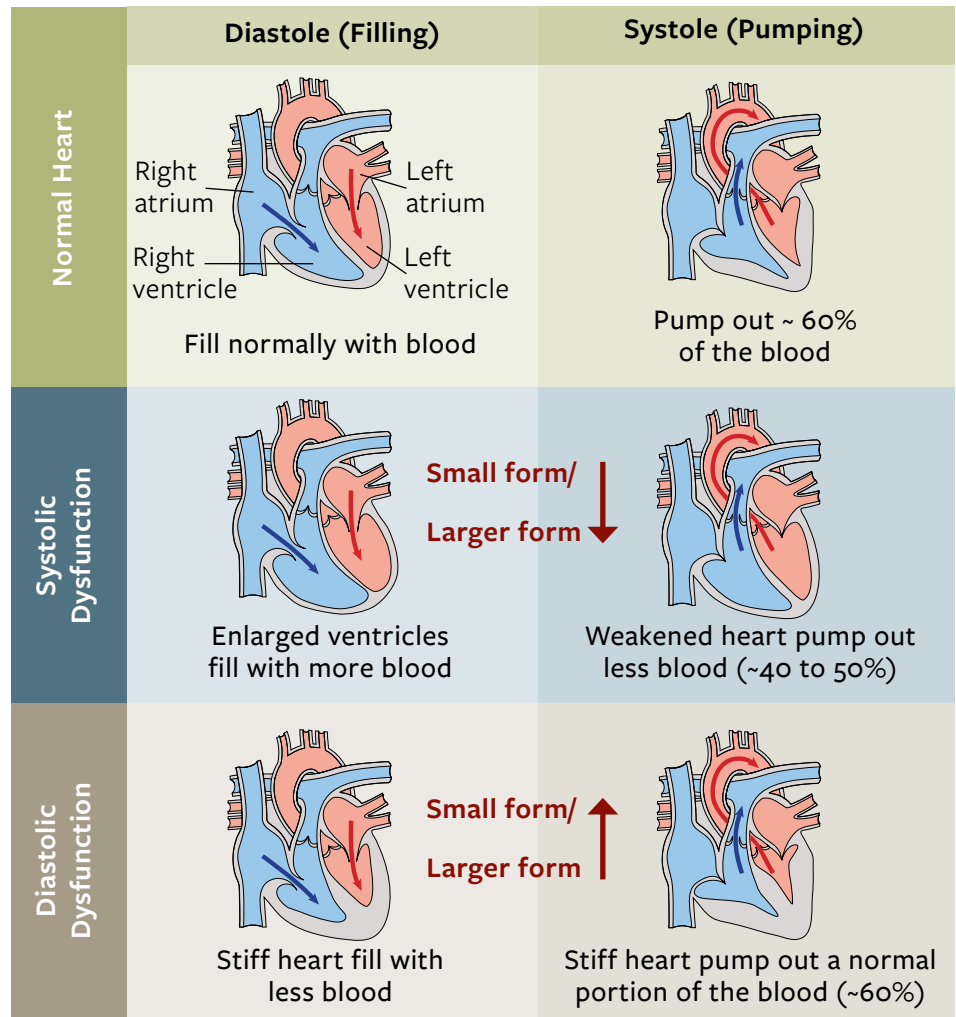
There are no treatment options to reduce heart wall stiffness for diastolic failure. Titin, due to its elastic property, has been considered as a promising therapeutic target to treat diastolic failure.

heart, the two titin forms can still be switched. For example, in the rodent model, we observed the larger form is expressed in the fetal heart, then gradually, the form is switched to the smaller type until it is the predominant one in the adult heart. However, without RBM20, no switching occurs during development (Figure 5B). These data suggest other factors could work with RBM20 to regulate the switching from one type of titin to the other.

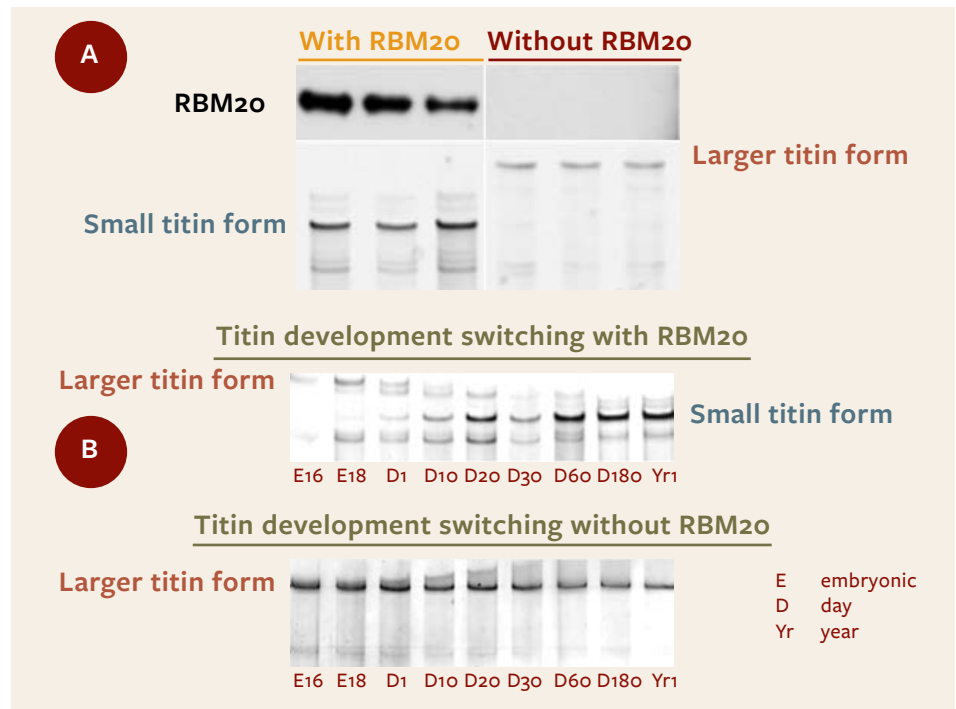
### On-going Work

To achieve our ultimate goal, the first step is to understand how RBM20 regulates the switching of the two types of titin. When we answer this question, we will be well on our way to discovering the tools needed to regulate heart wall stiffness as a novel way to treat diastolic failure.

**To contact:** Guo can be reached at (307) 766-3429 or at [wguo3@uwyo.edu](mailto:wguo3@uwyo.edu).



**Figure 4.** Effects of dysfunctions on heart efficiency



**Figure 5.** A. With RBM20, the smaller form of titin is expressed. Without RBM20, the larger form is expressed. B. Even when RBM20 is present, the two forms can be switched. The larger form is expressed and gradually is switched to the smaller type. Without RBM20, no switching occurs.

# The OTHER brucellosis

Though not as well-known as its cattle counterpart, this disease worries sheep producers

Sheep brucellosis affects 49 percent of flocks across the United States, caused by *Brucella ovis*, a bacterium closely related to *Brucella abortus* that causes abortions in cattle.

Unlike *Brucella abortus*, *B. ovis* lacks a wildlife reservoir and does not cause disease in humans.

*B. ovis* can result in substantial financial losses and is a major concern to sheep producers. Even though Wyoming is the fourth-largest producer of sheep in the United States and accounted for \$51.3 million in 2012 sales, no research had been done since 2001 (see related story about the national study) to determine the prevalence of *B. ovis*.

We wanted to find out the current status of the disease in Wyoming flocks.

This was a producer-driven study, and flocks included those we contacted directly or that sent samples in for testing at the Wyoming State Veterinary Laboratory during 2015-2016. Protecting the identity of producers was a major concern, as some counties only had two flocks tested. For this reason, results were grouped into five geographic regions (West, Northwest, Northeast, Southeast and South-central), each including four to five counties.

**Kerry Sondgeroth**  
Assistant Professor

**Molly Elderbrook**  
Graduate student,  
Department of Veterinary  
Sciences

## Briefly ...

Sheep brucellosis is a concern for many producers. While 49 percent of flocks in the United States in 2001 tested positive for *Brucella ovis*, only 22 percent of the flocks tested in Wyoming during 2015-2016 were positive. Possible factors that increase risk of *B. ovis* include larger flock size and higher ram-to-ewe breeding ratios.



We attempted to include flocks of all sizes from all five regions. Wyoming has approximately 293 flocks, with 20-100 flocks per region (USDA 2012 Census of Agriculture). A total of 82 flocks were used in this study. Flock sizes range from small (less than five sheep) to very large (more than 10,000 sheep). A flock was determined to be positive if at least one animal from that flock tested positive.

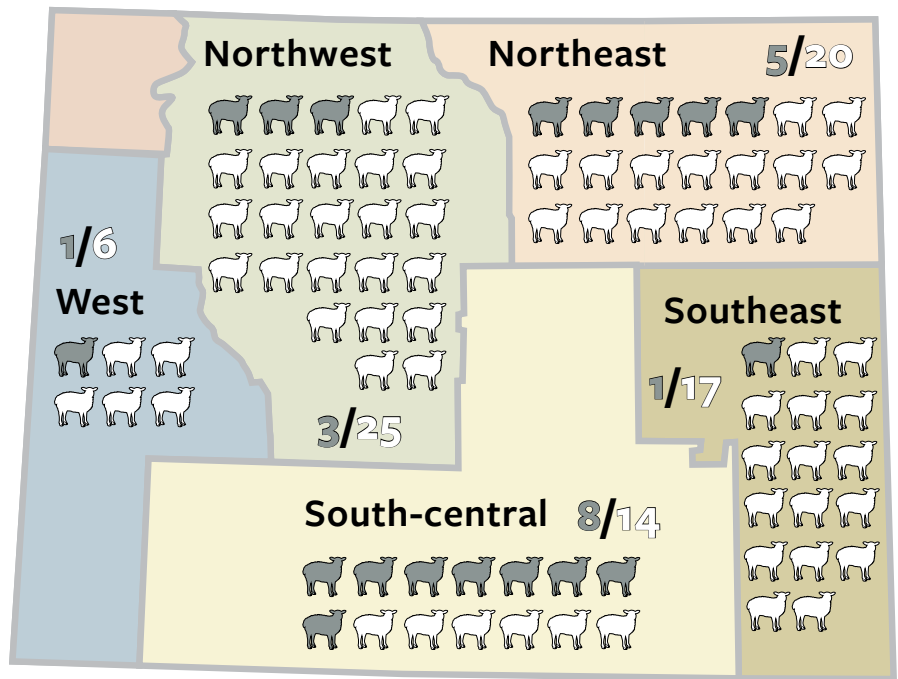
There was some variation in the number of positive flocks from each region, with the lowest at 6 percent (Southeast) to the highest at 57 percent (South-central), which had the highest proportion of flocks with at least one *B. ovis* positive animal. We are uncertain about what additional factors may contribute to this finding.

Overall, 22 percent (18/82) of tested Wyoming flocks were positive for *B. ovis* antibodies, which is much lower than the U.S. average (49 percent).

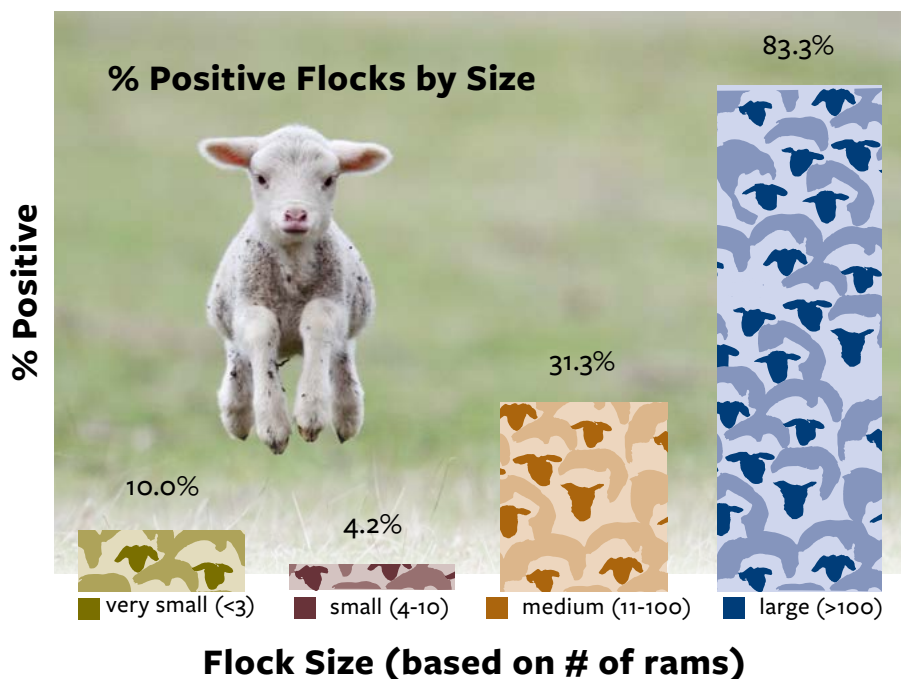
Flock size was classified based on the number of rams. There were 20 very small flocks, 24 small, 32 medium and 2 large. Similar to what we found in the nationwide study (see national study story), larger flocks, with more than 100 rams, had more *B. ovis* exposure in comparison to those flocks with less than 10 rams.

Future studies will focus on the diagnostic testing for this disease. We are comparing three different enzyme-linked immunosorbent assay (ELISA) tests (one currently available in the United States, one only available in Europe, and one currently in development), to determine if there may be a “better” test for diagnosing *Brucella ovis* in sheep.

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**Figure 1.** Geographic regions showing the number of flocks tested with the number of positive flocks in black.



**Figure 2.** #positive flocks/total # flocks based on size: vs=2/20, s=1/24, m=10/32, l=5/6

## 2001 study helps determine how many U.S. flocks have *Brucella ovis*

A team of undergraduates at the University of Wyoming in 2016 tested blood samples taken during a 2001 national study to determine how many flocks across the United States were affected by *Brucella ovis* (*B. ovis*), and if possible, identify risk factors associated with infection.

The number of sheep flocks affected by *B. ovis* across the United States was unknown prior to this project.

Blood samples from ewes across the Eastern, Central, West Central and Pacific regions of the United States were used. Wyoming was included in the West Central region (see our Wyoming results story page 20). A total of 676 flocks from 22 states participated as part of the National Animal Health Monitoring System Sheep 2001 Study. The blood samples were collected for health assessments and disease monitoring, and unused portions were frozen for long-term storage. This provided over 16,000 sheep samples for testing.

Blood samples were tested for antibodies to *B. ovis* at the Wyoming State Veterinary Laboratory in Laramie using an enzyme-linked immunosorbent assay (ELISA). The undergraduates were taught how to perform the ELISA, so multiple samples could be tested each day during the summer of 2016.

### What Did We Find?

From the 676 flocks, 49 percent of them had at least one ewe test positive for antibodies. This means almost half of the sheep flocks in the United States have been exposed to *B. ovis*. If we look at the number of positive flocks per region, the West Central has the highest with 59 percent, and the Eastern region has the lowest with 34 percent.

### What is Different about *Brucella ovis* Positive and Negative Flocks?

While some geographic regions of the United States certainly seem to have more *B. ovis*, this is likely due to flock sizes and the way in which flocks are managed in these different regions. Larger flocks (more than 100 ewes and lambs) are associated with more exposure to *B. ovis*, but this could be due to grazing practices on open range versus farm flocks that have no exposure to other flocks.

The breeding ratio of ram to ewes is also associated with increase in *B. ovis*, and this may be due to the importance of indirect transmission. There is a lower proportion of *B. ovis* positive ewes in flocks that breed 1 ram to 19 or fewer ewes.

### Why is this important?

This is the first nationwide study of *Brucella ovis* in the United States. This provides producers and veterinarians information about a potential bacterium that could be causing reproductive problems in their flocks.



*Brucella ovis* causes reproductive problems such as ram epididymitis, and while ewes do not show signs of infection, it is associated with a higher number of open (non-pregnant) ewes and occasional abortions. Rams are the primary way of transferring the bacteria to other ewes during the breeding season. An infected ram can pass the infection onto ewes, and the ewes can then infect another ram if bred again. This is referred to as indirect transmission. However, rams can also pass the infection to other rams, and this is referred to as direct transmission.

### How was this project possible?

The study was part of graduate student Molly Elderbrook's master's degree project. We are grateful for the participation of the Wyoming producers during 2015-2016, and the USDA for allowing us to test their samples. We utilized funding from multiple sources, including grants from the Wyoming Agricultural Producer Research Grant and the Wyoming Agricultural Experiment Station, and a cooperative agreement with USDA-National Animal Health Monitoring System.





# PROTECTING THE HERD:

## Child immunization in Wyoming

Vaccine hesitancy noted in some counties for measles, mumps, rubella; polio in others

**Mariah Ehmke**  
Associate Professor  
Agricultural and Applied Economics,

**Chen Xu**  
Assistant Professor  
Department of Geography,  
University of Wyoming

Outbreaks of preventable childhood diseases over the last few years have highlighted the risk of declining herd immunity in some areas of the country. Herd immunity (see page 26) helps prevent the spread of infectious diseases when a high proportion of people are immune to those diseases, typically through vaccination.

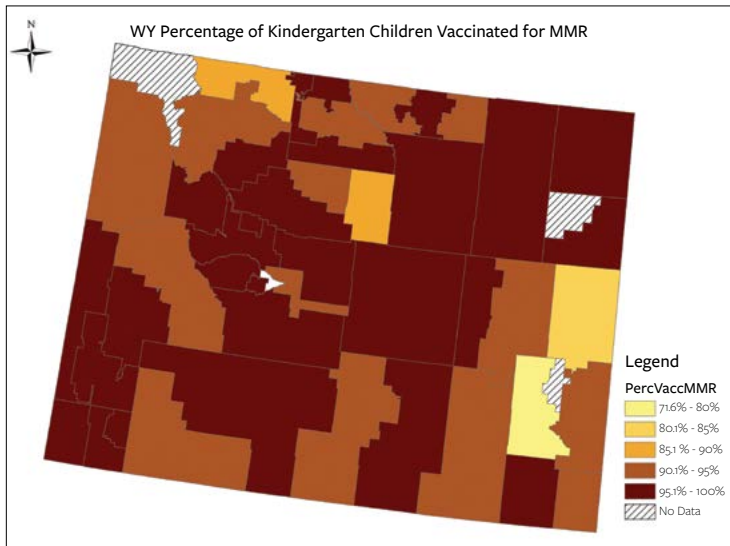
In 2015, nearly 70 cases of measles

were confirmed in California following an outbreak at Disneyland. The risks of preventable disease outbreaks in rural areas such as Wyoming may seem remote; however, when compared to other states, statistics suggest Wyoming's young children may be at risk to disease outbreaks.

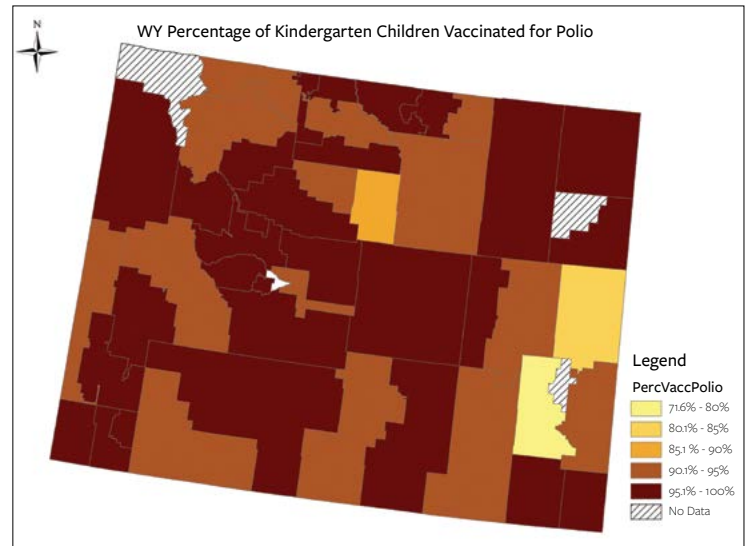
We analyzed immunization rates across Wyoming communities to



[https://youtu.be/WzxQ\\_Fq8ewA](https://youtu.be/WzxQ_Fq8ewA)  
<https://youtu.be/7ysxZhUSSjc>  
<https://youtu.be/pK-C8ArrzLg>  
<https://youtu.be/4X--EIDalmo>



**Figure 1.** Percentage of kindergarten children vaccinated for measles, mumps, and rubella (MMR)



**Figure 2.** Percentage of kindergarten children vaccinated for polio

determine if there are weak areas of herd immunity and found significant differences in immunization rates across schools of different sizes. There is also unexplained variation in the percent of students immunized for specific diseases across the state.

### Data from 197 Wyoming Schools

We used 2016 kindergarten registration data from 197 schools provided by the Wyoming Department of Health's Immunization Unit to determine what areas of the state are below or meet the Healthy People 2020 goal of a 95 percent immunization rate.

The data are from primary school reports of the number of kindergarten students entering kindergarten with complete, partially complete, or no immunizations.

Overall, our data show children in the statewide kindergarten age group are typically more up-to-date on immunizations than those in the Centers for Disease Control and Prevention's (CDC) study of 19 to 35 month olds.

The types of immunizations include diphtheria, tetanus, pertussis, measles, mumps, rubella, hepatitis B, and varicella (chicken pox). It is possible, however, children are allowed to attend kindergarten without these immunizations if their parents list a religious objection to immunization. No questions are asked, but the parents are often notified their child is not fully immunized.

Wyoming kindergarten children are close to meeting Healthy People 2020 goals on average, according to 2016 data. Overall, our data show children in the statewide kindergarten age group are typically more up-to-date on immunizations than those in the Centers for Disease Control and Prevention's (CDC) study of 19 to 35 month olds.

Over 95 percent of kindergarten students were immunized against hepatitis B. For other immunizations, the proportion of immunized children ranged from an average of 93.6 percent for diphtheria to 94.3 percent for polio. The average number of kindergartners enrolled at each school was 38.8.

We analyzed the data using two methods.

First, we statistically tested whether school location or class size had an effect on the number of children vaccinated

using a simple linear regression model.

Second, we used geographical thematic maps to show the variation in immunization rates across Wyoming school districts. (See Figures 1-7.)

### School Size a Factor

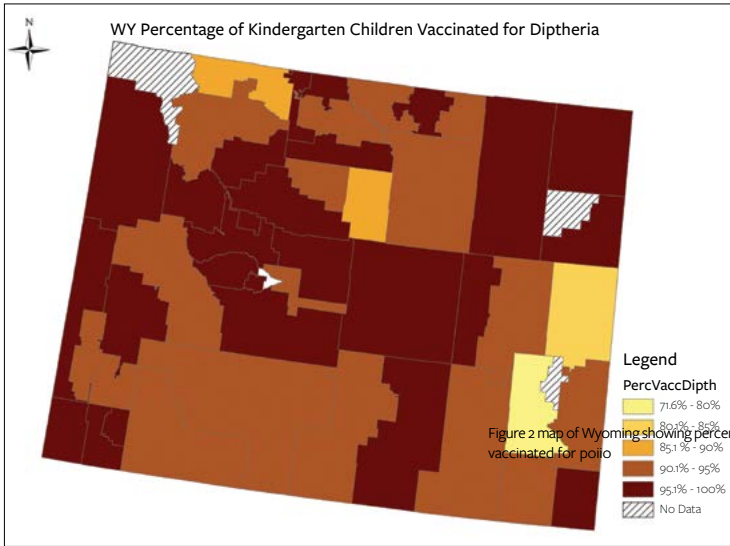
We found significant differences in immunization rates across schools of different sizes. On average, if a school counts one additional child among their kindergarten students, they will have a 0.07 percent increase in the proportion of immunized kindergartners. To the degree school size reflects the community population, this indicates children in more rural areas of the state may be less likely to be fully immunized.

There is still unexplained variation in the percent of students immunized across the state for measles, mumps, and rubella (MMR) and polio.

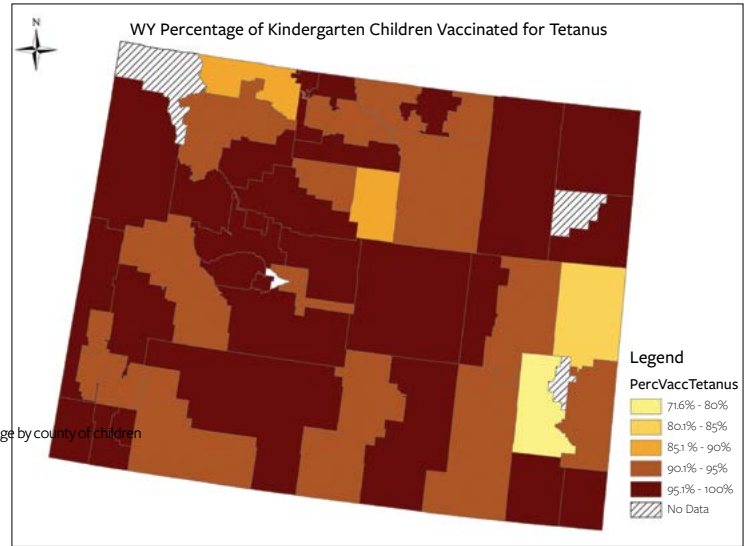
We turned to geographic maps to illustrate some of this variation. School district level immunization rates for MMR and polio are illustrated in Figures 1 and 2. Comparing the two figures, there are more areas of vaccine hesitancy for MMR than polio. The decline in immunization rates for MMR, compared to polio, may be an indication of greater hesitancy in areas around Teton,

*(Continued page 26)*

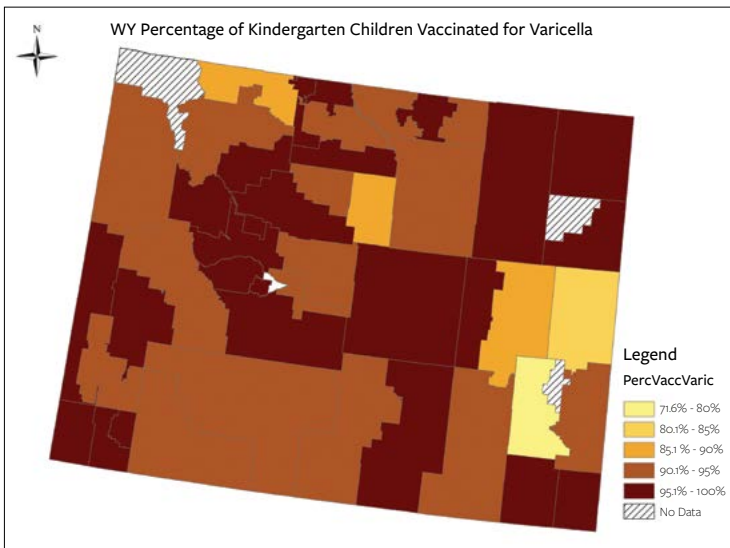




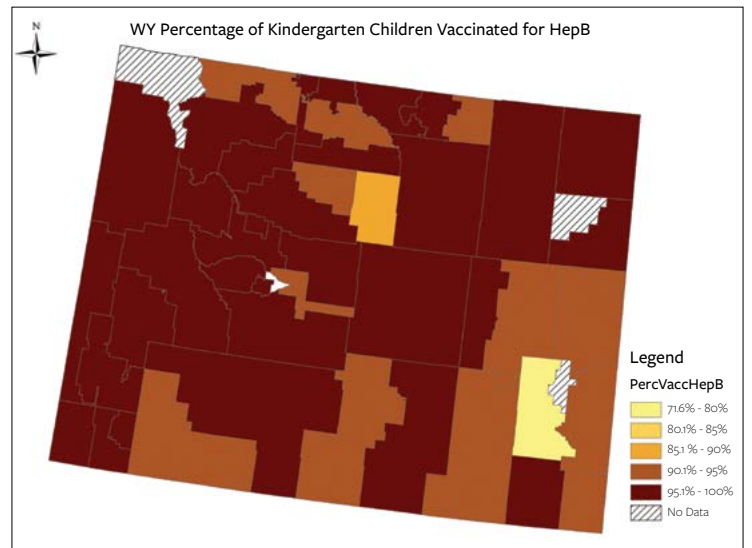
**Figure 3.** Percentage of kindergartners vaccinated for **diphtheria**



**Figure 4.** Percentage of kindergartners vaccinated for **tetanus**



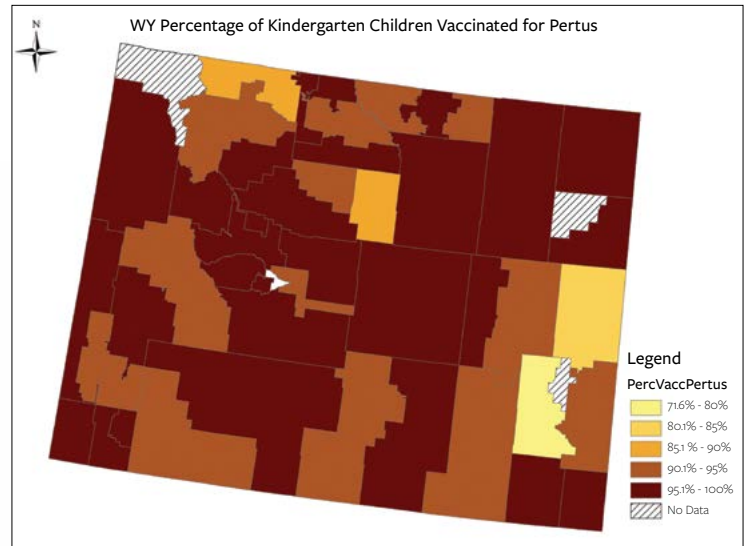
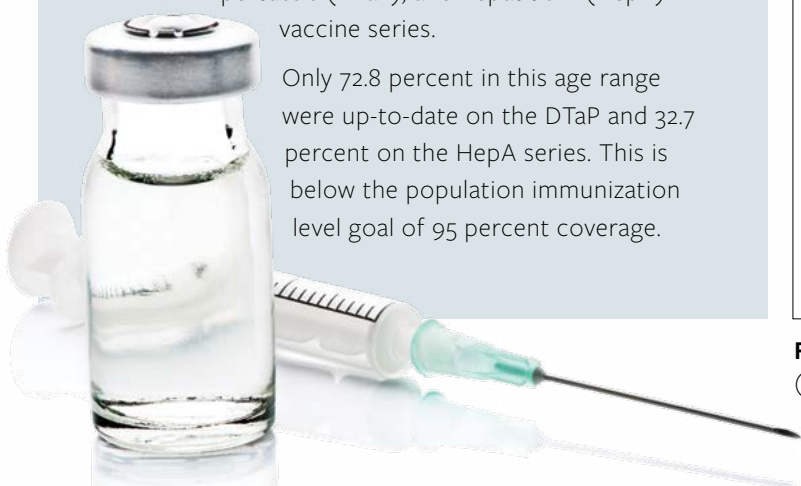
**Figure 5.** Percentage of kindergartners vaccinated for **varicella** (chicken pox)



**Figure 6.** Percent of kindergartners vaccinated for **hepatitis B (HepB)**

Epidemiologists at the Centers for Disease Control in 2015 reported Wyoming had the lowest reported immunization rates in the nation for children aged 19 to 35 months for the diphtheria, tetanus, and pertussis (DTaP), and hepatitis A (HepA) vaccine series.

Only 72.8 percent in this age range were up-to-date on the DTaP and 32.7 percent on the HepA series. This is below the population immunization level goal of 95 percent coverage.



**Figure 7.** Percent of kindergartners vaccinated for **pertussis** (whooping cough)

Laramie, Big Horn, Park, and Sheridan counties in particular.

While more children were immunized against polio than MMR in most areas of the state, this is not true for parts of Lincoln, Johnson, and Sheridan counties. There we observe slight decreases in polio vaccination compared to MMR rates.

This deeper look at Wyoming kindergarten immunization data suggests many children do “catch up” on the immunization series by kindergarten. This age group is not at as great a risk as the 19 to 35 month olds in the earlier CDC data.

### Further Research

Parents and public health officials in the state do need to be aware children in more remote and smaller schools may have increased risk of disease exposure, and there is a need to examine causes of vaccine hesitancy and avoidance in certain areas and for specific vaccines.

Associate Professor Mariah Ehmke is working with a team of graduate and undergraduate students to examine behavioral economic dimensions of parents’ decisions to immunize their children in different communities. Their work focuses on parents’ adherence to social norms, perceptions of risk, and use of medical information.

This research is funded by the National Institute of Health’s Mountain West Clinical and Translational Research-Infrastructure Network. Results of the work may provide guidance to public health and medical professionals communicating vaccine and disease risk to parents.

**To contact: Ehmke** can be reached at (307) 766-5373 or at [mariah.ehmke@uwyo.edu](mailto:mariah.ehmke@uwyo.edu). **Xu** can be contacted at 307-223-5686 or at [cxu3@uwyo.edu](mailto:cxu3@uwyo.edu).

## What is herd immunity?

Immunization supports herd or community immunity when a high proportion of members of a community are not likely to catch a contagious disease.

If a high enough proportion of individuals are immunized and not susceptible to infection, disease outbreaks may be prevented and even individuals ineligible for immunization receive protection.

The diagram below illustrates disease transmission through a community with and without herd immunity. None of the population members are immunized in the upper Area A. A new disease enters from the left and travels through the population affecting everyone. One person is affected in the first generation of the disease. That person then infects three people. And then, those three people each infect three more people and so on.

In Area B, in the lower level of the figure, about two-thirds of the population is immunized. In this case, when the person on the left brings in the disease, they only infect one person. That person then infects one of the three people with which they had contact.

In this hypothetical situation, immunization decreases the number of infected persons from 13 in Area A to 3 in Area B or results in a 77 percent decrease in infection rate. Two members of the herd in Area B are not immunized. They may represent people who aren’t able to receive immunization because of medical disorders or conditions such as pregnancy.

Still, they do not contract the disease because other herd members are immunized.

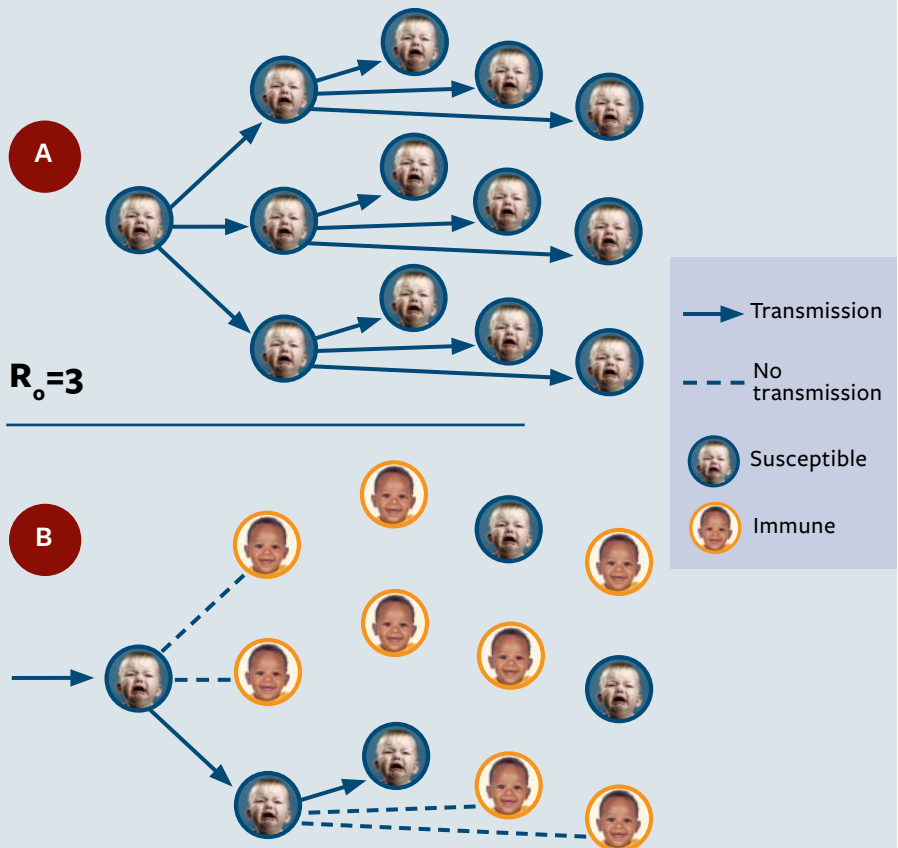


Diagram of transmission outcomes across two communities without and with herd immunity. The diagram is taken from the National Academies of Science Committee on the Assessment of Studies of Health Outcomes Related to the Recommended Childhood Immunization Schedule and Board on Population Health and Public Health Practice (2017).



# Tracking agricultural water in Wyoming: Quantifying return flows to streams



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*Hydrologic Sciences*

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*Professor and head*

*Department of Ecosystem Science  
and Management*

**Andy Parsekian**

*Assistant Professor*

*Department of Geology and  
Geophysics and Department of Civil  
and Architectural Engineering*

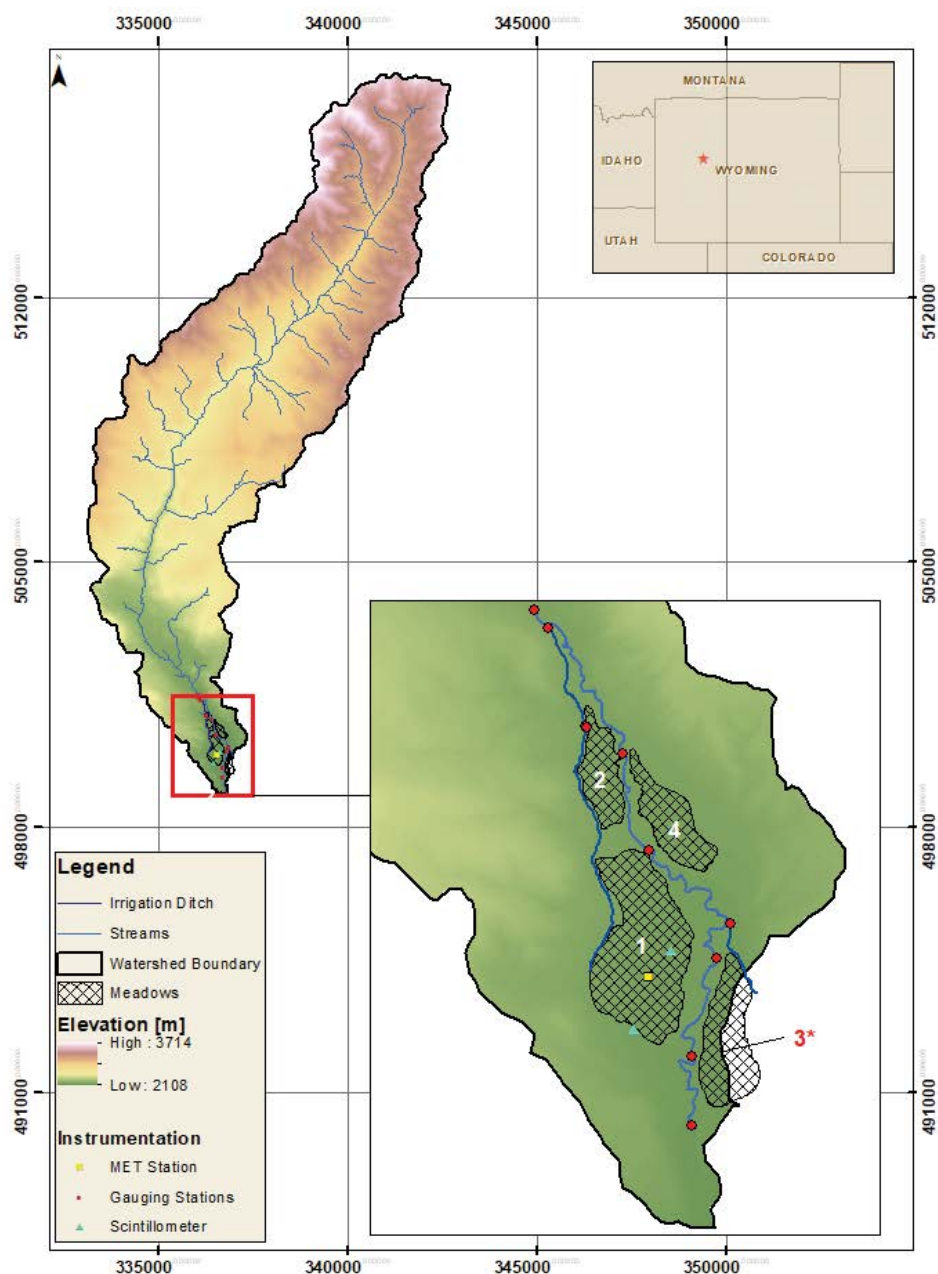
## High-tech tools gaze below soil surface to track and measure water

Water management in the western United States and Wyoming in particular has much in common with real-estate markets – it is significantly influenced by timing and location.

Most water in Wyoming comes from spring snowmelt flowing from high alpine mountains down to the shrub and grasslands lower in the watersheds, and over 70 percent of available surface water comes from snowmelt in May and June.

In 2015, researchers from the Wyoming Center for Environmental Hydrology and Geophysics (see at [uwyo.edu/epscor/wycehg/](http://uwyo.edu/epscor/wycehg/)) initiated a project to determine the quantity and time of return flow in Bear Creek, a tributary of the East Fork of the Wind River (Figure 1), and to track near surface flow processes.

The project is in collaboration with the Wyoming Game and Fish



**Figure 1.** Location of Bear Creek Study area in the Upper Wind River Basin with detailed locations of hydrologic instrumentation and irrigation fields. Field 3 does not contribute return flow to Bear Creek.

Department, which manages irrigation on Bear Creek as part of the Spence-Moriarty Habitat Area, and was funded in part by the Water Resources Program at the University of Wyoming.

Our expectation was that return flow in this watershed would be different (most likely less) than the New Fork (described in “Mid- to late season flows” page 31). It has very different soils and

watershed characteristics: thick, sandy loam soil over cobbly, glacial till.

For three years we have used a combination of traditional intensive, hydrologic observations (rainfall, stream flow, and irrigation application) coupled with geophysics tools that allow us to track water storage and movement to approximately 10-15 meters below the surface.

We found about 38 percent of the

applied water returned to Bear Creek – less than the return flow observed in the New Fork (Figure 2). We also found most of the return flow occurred during the irrigation season and most likely does not contribute to late-season flows in the Wind River Basin.

So by which pathways does the applied irrigation water that is not consumed by plants return to the stream?

Possible pathways include:

- 1) surface flows,
- 2) subsurface lateral flows, and
- 3) flows into groundwater connected to the stream (Figure 3).

### Geophysics Tools Track Water

In addition to measuring the agricultural water balance (see “Water balance” page 29), we have conducted intensive field studies during irrigation in Bear Creek using two geophysics methods: electrical resistivity tomography (ERT) and nuclear magnetic resonance (NMR) to track subsurface water movement and calculate storage changes, respectively.

Geophysics uses tools that react to the physical properties of materials found in the soil allowing researchers to “see” and track water movement below the surface.

ERT measures resistance to an electrical current (resistivity) in the subsurface using electrodes installed on the soil surface.

The distribution of resistivity is a function of many characteristics of the subsurface (for example, mineralogy, chemical composition and water content); however, if measured over time during irrigation, only changes in water content will cause changes in resistivity.

Because of this property, we can use time-lapse ERT to track changes in water content and map flow dynamics during

*(Continued page 30)*

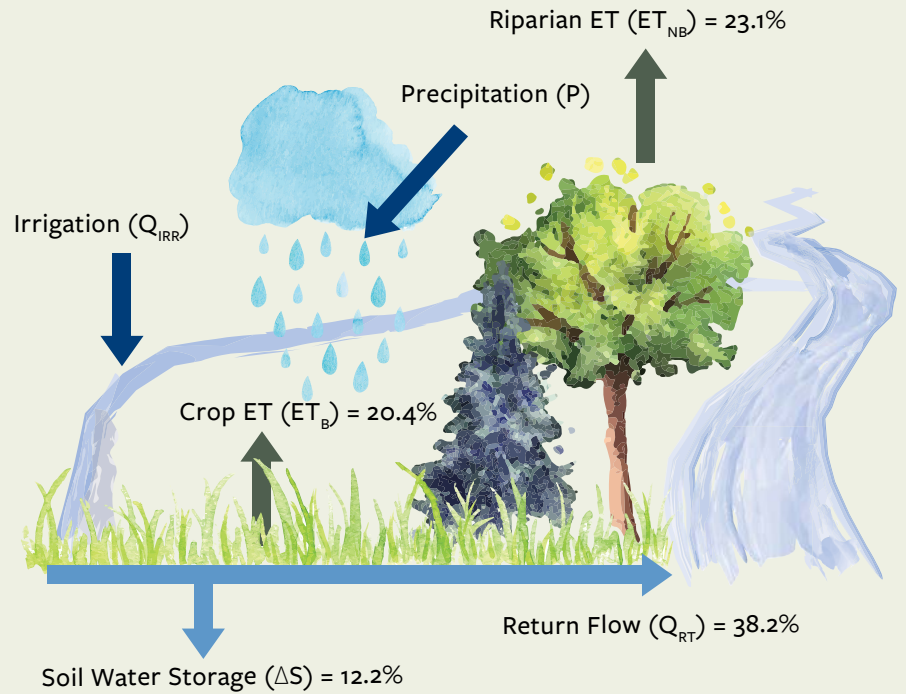


## Finding a water balance

An agricultural water balance is an intensive but common sense approach to quantify the potential amount of water that would or could return to Bear Creek (see main story).

We measured or estimated all of the hydrologic components, inputs and outputs, within the study area during the irrigation season (see Figure 2). The inputs into the system include precipitation (P) and applied irrigation water ( $Q_{IRR}$ ), and the outputs include evapotranspiration (ET) of both the crop (beneficial) and riparian vegetation (non-beneficial), and changes in water storage ( $\Delta S$ ).

Coupling these measurements with dense measurements of stream flow during the irrigation season (seven gauging stations were installed along Bear Creek) allowed us to close the water balance and quantify the amount of return flow. In this way, we were able to account for almost 94 percent of the water in this particular system.



**Figure 2.** Results of solving the agricultural water balance for Bear Creek. We closed the balance to 93.9 percent, the remaining 6.1 percent is error and uncertainty.

## Instrumentation & Measurements to Calculate Water Balance

The Agricultural Water Balance can be written as:

$$(P+Q_{IRR}) = Q_{RT} + (ET_B + ET_{NB}) + \Delta S$$

**Precipitation (P)** was continuously recorded using a tipping bucket rain gauge. Instrument accuracy was +/- 1% up to a rate of 1 inch per hour.

**Stream flow (Q)** was measured at seven gauging stations established along Bear Creek (see Figure 1). Gauging stations were strategically located to capture return flow from each of the adjacent agricultural meadows as well as to establish clear points of inflow and outflow. Manual flow measurements were made using a flow meter to capture a range of flows and develop a “rating-curve” that relates our continuous stream depth measurements from the gauging stations with measured flows in the stream for all sites. The study reach of Bear Creek between the inflow and outflow gauges is roughly 3.05 km.

**Applied Irrigation ( $Q_{IRR}$ )** was measured using pressure transducers in the irrigation ditch.

**Return Flow ( $Q_{RT}$ )** was calculated in two ways. First, by solving the water balance equation and second by calculating changes in stream flow within the channel using the seven gauging stations.

**Soil water storage ( $\Delta S$ )** was measured using soil moisture probes installed at 0.30 m, 0.60 m, and 0.90 m depths during the irrigation season.

Deeper soil water storage was measured using surface **Nuclear Magnetic Resonance (NMR)** soundings on the agricultural fields. NMR soundings were conducted before and after the irrigation season to detect changes in groundwater storage up to 15 meters in depth.

**Net evapotranspiration** was calculated using a combination of direct measurements and a physically based model. Beneficial **crop evapotranspiration ( $ET_B$ )** from agricultural areas was measured directly on the irrigated fields and the non-beneficial **evapotranspiration ( $ET_{NB}$ )** from adjacent riparian areas estimated using meteorological observations and the model.

irrigation (wetting) and following irrigation (drying). (Figure 4) We have also been able to track the movement of the wetting front in the subsurface and identify flow directions (Figure 5).

NMR measures subsurface water content by emitting an electro-magnetic pulse that targets hydrogen atoms in water. The resulting signal generated by the hydrogen atoms is proportional to the volume of water in the subsurface. The signals are measured by the instrument providing an estimate of soil water content by depth.

We use two different types of NMR: surface NMR to measure deep changes in water storage before and after the irrigation season; and borehole NMR in conjunction with time-lapse ERT during irrigation to directly measure changes in soil water content.

### What's Next?

Putting all of the pieces together is the next step. We have made great strides over the last three years quantifying the return flow in Bear Creek. Over the coming months we will use the information we've gathered to construct hydrologic models. Modeling will allow us to directly relate the characteristics of the Bear Creek watershed to observed hydrologic processes. It will also allow us to evaluate these relationships in other Wyoming irrigation districts to hopefully better predict water availability throughout the year.

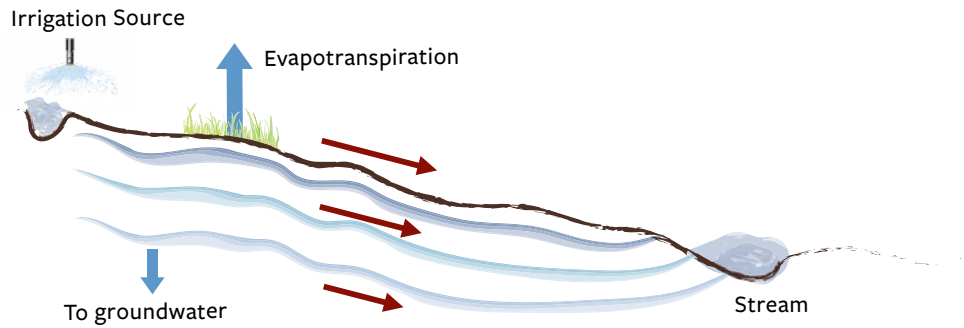
**To contact: Paige** can be contacted at (307) 766-2200 or at [gpaige@uwyo.edu](mailto:gpaige@uwyo.edu).



<http://bit.ly/summerfieldphotos>



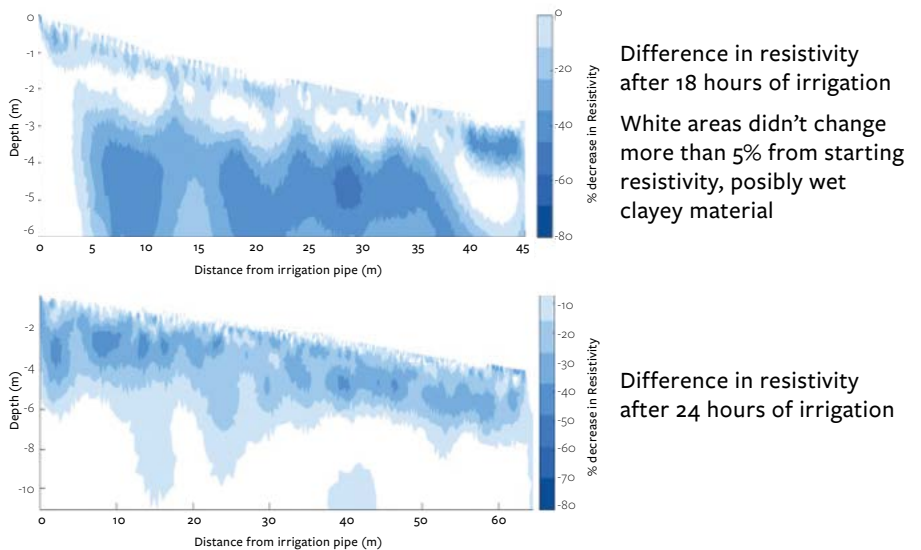
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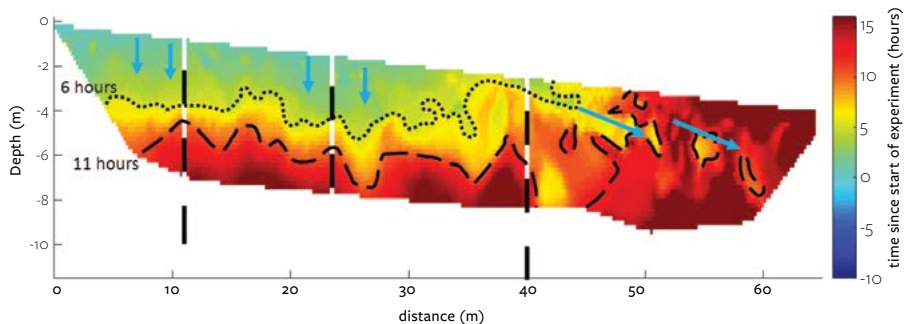
Three different pathways (red arrows):

- Surface runoff
- Subsurface runoff/vadose zone
- Return flow via deeper aquifers

**Figure 3.** Three different possible return flow pathways.



**Figure 4.** Tracking changes in field-scale water movement in the subsurface during irrigation (wetting and drying) using ERT.



**Figure 5.** Time of wetting front arrival and direction of flow during irrigation. The three vertical (black and white) lines represent the locations of the three bore holes along the transect used. The blue lines indicate the direction in which the wetting front advances.





The time-lapse electrical resistivity tomography (ERT) equipment is laid out in a field in the Bear Creek Study area. ERT measures the electrical current (resistivity) in the subsurface using electrodes installed on the soil surface.



## Mid- and late-season flows

Wyoming surface water is primarily sourced from spring snowmelt. So how do we end up with water in some Wyoming streams in the late summer and fall?

In some watersheds, perennial flow in streams is maintained from groundwater connectivity. In many cases, agricultural use holds or retains water in watersheds during the early-to mid-summer agriculture season and then “releases” the unused portion.

Flood irrigation, although not the most water-efficient method, is often the most cost-efficient for lower economic-value crops such as hay and forage and is widely used in Wyoming.

Irrigation water delivered to a field or a section of a field via pipes or ditches often saturates the upper portion of the soil profile. Water not used by crops or other vegetation either moves deeper into the soil and potentially into groundwater, or it can return via surface or near subsurface flow to an adjacent stream.

In Wyoming, water managers generally assume about half of applied flood irrigation water returns to an adjacent stream. A study in the Upper Green River Basin, in the New Fork Irrigation District, found 70 percent of water applied returned to the stream and 10 percent returned as late-season (August-September) flow.

The characteristics of the New Fork Irrigation District may be unique – it has shallow soils underlain by a gravelly, shallow aquifer that can take and hold irrigation water and then slowly release it back to the New Fork River. Not all basins, or irrigation districts, have these same characteristics.

Two driving questions are:

- 1) what is the range of potential return flow in Wyoming? and
- 2) can newly available tools and approaches be used to quantify the volume and timing of return flow and identify the flow paths (surface and sub-surface)?

# GOING GREEN



## On the road to environmentally safer grasshopper control

**Lee Noel**  
Master's student  
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Entomologist  
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Management

For some, seeing grasshoppers peacefully springing through fields may elicit sentimental memories of a childhood spent playing.

For others, it's enough to induce sheer terror.

Love them or hate them, grasshoppers have been around for millions of years, and they continue to shape rangeland management and crop production.

For decades, ranchers have relied exclusively on chemical insecticides to control pest grasshoppers on rangelands surrounding the Rockies. Millions of rangeland acres must be blanketed with insecticides during grasshopper outbreak years, causing health hazards to humans and wildlife.

This “friendly fire” can be especially deadly to beneficial pollinator insects such as bees and butterflies because the chemicals are non-selective—they are *insecticides*, not *grasshoppercides*.

A more environmentally responsible approach began growing in the United States as the perils of overusing pesticides became apparent. This opened the door for researchers at the University of Wyoming to start looking for novel, environmentally friendlier, and more

sustainable ways of managing those pesky grasshoppers.

### The Beginning

Professor Alexandre Latchininsky, master's student Lee Noel, Ph.D. candidate Douglas Smith, and extension entomologists John Connett and Scott Schell have been developing a new anti-grasshopper recipe: a bait mixture of wheat bran and a lethal microbial pathogen.

Specifically, they have been testing the effectiveness of different fungi, which occur naturally in North American soils and produce spores that can kill grasshoppers without affecting non-target organisms. After choosing the naturally occurring strain DWR2009 of *Metarhizium robertsii* fungus as the potential biological control agent, two main questions remained for the researchers: what is the most effective dose rate of the bait, and how long do pest grasshoppers need to be exposed to the bait to kill them?

This journey to the “green” grasshopper control began in the summer of 2015, when the entomologists found themselves in Lusk catching around 1,200 grasshopper nymphs. Plenty of test subjects are needed to carry out



replicated research! *Melanoplus sanguinipes* and *Melanoplus differentialis* are known as two of the most devastating species in the West, eating about half their body weight in a day and causing significant forage destruction when their populations reach outbreak numbers – above 30 per square yard.

These hoppers were transported to the insectary of the Department of Ecosystem Science and Management in the College of Agriculture and Natural Resources and raised to adulthood.

The wheat bran bait mixture infused with canola oil and fungal spores was prepared with the help of renowned grasshopper pathologist Stefan Jaronksi from the United States Department of Agriculture. Different doses of bait were placed in the field and “cages,” made from sour cream containers with holes drilled in them and covered in mesh netting, were put on top. A single grasshopper was placed into each cage, exposed for three days to the bait, and then brought back to the lab for two weeks of observations, noting the day when each hopper died.

Five field experiments were carried out in this fashion, mostly resulting in disappointingly low grasshopper mortality (10 percent).

What went wrong?

### We Modify our Tests

Researchers went back to the drawing board as winter arrived in Laramie. Considering their results and taking suggestions from scientists at the National Grasshopper Management Board meeting after presenting the data, two changes were implemented: increase exposure time to the bait to three and six days, and increase the dosages of bait to 5, 10, and 20 pounds/acre,



Each plastic container covered one grasshopper and a specific dose of wheat bran bait.

which are similar to insecticidal bait recommendations.

Experiments were moved indoors and conducted in climate-controlled cabinets. With drastic increases in mortality rates (above 50 percent in most cases), the researchers knew the product worked in an ideal environment, but it was time to take their experiments to the next level.

Noel spent the summer of 2016 at the USDA Agricultural Research Service Northern Plains Agricultural Research Laboratory in Sidney, Montana, working with Jaronksi, a respected grasshopper expert and great mentor. Together with lab manager Rob Schlothauer, they made new batches of the fungus-infused wheat bran bait, and Noel learned the tricks of the trade when it came to raising *M. sanguinipes* and *M. differentialis* grasshoppers.

Aside from a brief organic lettuce shortage (the hoppers’ favorite food), the expansive research facilities in Sidney allowed production of hundreds of grasshoppers every two weeks, and grasshopper egg pods could be continually collected from adult females.



Indoor experiments increased wheat bran bait dosages and grasshopper exposure times.

**Success!**

Nine experiments were conducted over the summer. Six experiments took place indoors in climate-controlled rooms using nymph grasshoppers of both species. These smaller, young grasshoppers were hypothesized to be more susceptible to the fungus than larger adults, and the researchers were interested in the possibility of targeting the grasshoppers at earlier life stages.

The cages contained one grasshopper per each and were placed on artificial turf (like athletic fields) to simulate field conditions. Then a specific amount of bait (5, 10, or 20 lb/acre dose) was administered and the grasshoppers were exposed for either 1, 3, or 6 days, using up to 130 hoppers per treatment.

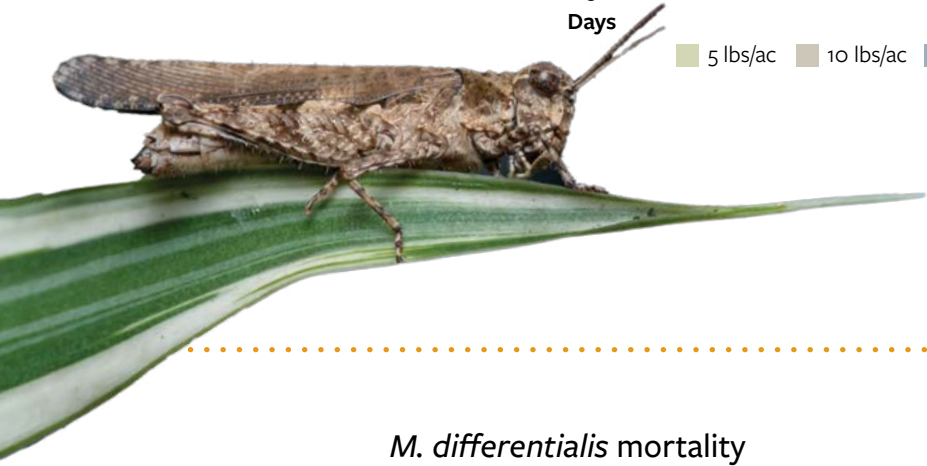
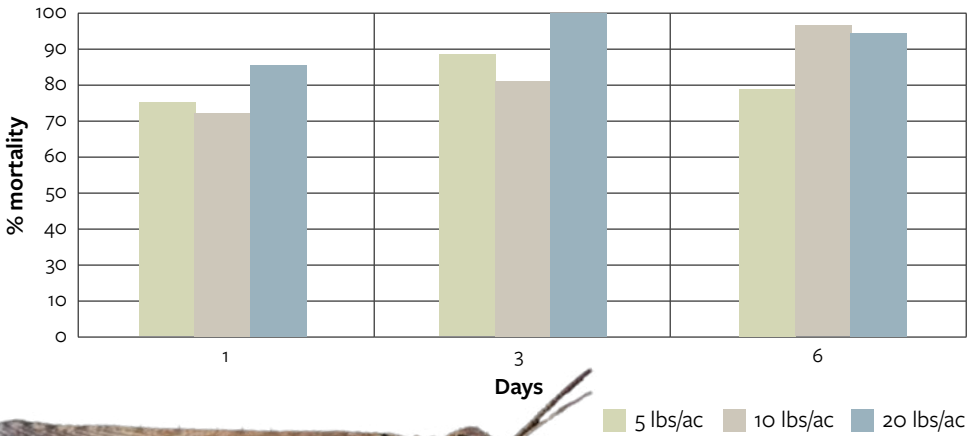
The experiments were a success this time: our bait was indeed lethal causing high mortality rates in most treatments. Two weeks post-exposure monitoring determined that, as expected, the 20 lb/ac treatment caused the most mortality in *M. sanguinipes*.

Intriguingly, the outcome was different with *M. differentialis*, where even the medium 10 lb/ac treatment caused near-identical mortality as the 20 lb/ac treatment. Analysis of exposure times showed *M. sanguinipes* grasshoppers experienced highest mortality rates when in contact with the bait for either 3 or 6 days. Interestingly, *M. differentialis* did not demonstrate any differences in mortality between the 1-, 3-, or 6-day exposure periods.

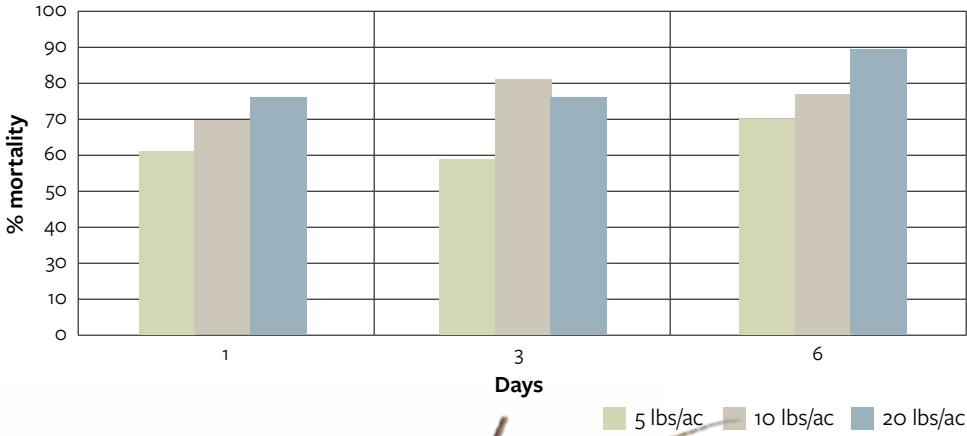
Further, *M. differentialis* grasshoppers experienced significantly lower mortality than *M. sanguinipes* (73 percent to 86 percent, respectively) and survived longer than *M. sanguinipes* (10.6 vs. 8 days).

Researchers hypothesized the larger size and body mass of *M. differentialis* grasshoppers made them more resistant to the fungus than the smaller

*M. sanguinipes* mortality



*M. differentialis* mortality





Love them or hate them, grasshoppers have been around for millions of years, and they continue to shape rangeland management and crop production.

*M. sanguinipes*. Also important was finding out that the longest exposure times and highest dosages do not always lead to the highest mortality.

#### What's Next?

Taking these experiments back out into the field is important to test the bait under real-world conditions.

Preliminary results from three field trials in Montana produced a much higher mortality than expected. These encouraging early tests have laid a solid foundation for further research. Another avenue of study will be exploring the potential side-effects of the fungal pathogen on non-target organisms to confirm it is indeed specific to pest grasshoppers and has very low, if any, negative environmental impact.

Knowing the most effective dose and exposure time could help ranchers save time and money using the bait. Furthermore, these results show the potential to move toward more environmentally friendly and sustainable options to control rangeland pests.

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Fungus-covered dead grasshopper

## WORKING FROM WITHIN: How fungus exterminates grasshoppers

UW scientists needed a fungus known to be lethal to grasshoppers, occurred naturally, and could be easily cultivated in a lab.

Enter *Metarhizium robertsii* strain DWR2009, which satisfied each requirement and had never been tested in a bait formulation. The strain's high heat tolerance is important because grasshoppers have the ability to fend off fungal infection via a behavior known as thermoregulation, where they use the sun's rays to increase their body temperature to a level that normally inhibits and eventually kills the development of spores inside them.

Entomopathogenic (specific and lethal to insects) fungi have a unique mode of action. Unlike bacteria and viruses, which need to be ingested to cause disease, fungal infection begins when the spores contact the cuticle (exoskeleton) of a susceptible host. The spores attach themselves to the grasshoppers' mouthparts as they munch on the bait.

Next, spores penetrate the cuticle of the grasshopper and start growing arm-like extensions called hyphae. The hyphae eventually extend into the abdominal cavity, where they begin to multiply. There, they deplete the host's nutrients and release toxins into the bloodstream that eventually kill the grasshopper.

Upon death, if the humidity around the grasshopper is near 100 percent, spores will begin to grow out of the grasshopper's body and on the surface of its skin, producing its reproductive stage – conidia – making for an interesting scene as the grasshopper appears to be covered in fuzz!



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