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Editor
Steven R. Chipps

Terri Symens, Wildlife & Fisheries, SDSU
Secretarial Assistant

Tom Holmlund, Graphic Designer
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Presented at the 92nd Annual Meeting of the South Dakota Academy of Science

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The Executive Council met at 12:00 pm Friday 13 April 2007 for a final check of plans for the symposium and meeting.

President Jim Sorenson opened the executive committee meeting and noted that a quorum was present.

Nels Troelstrup, Jr. reported estimated attendance for the banquet this evening and lunch tomorrow.

A copy of the Treasurer’s Report was distributed by Treasurer Kristel Bakker. The Audit Committee will consist of Audrey Gabel and Mark Gabel.

Upcoming meetings are: 2008 Cedar Shore Resort hosted by Mount Marty College; 2009 host NSU, contact to be determined.

Committee reports were as follows:

Publicity: First Past President Bob Tatina handled publicity for the 2007 meeting. He sent an electronic photograph to Neil Reese for posting on the SDAS website, phoned local TV stations about the upcoming symposium, e-mailed press releases to the Argus Leader, and coordinated external publicity on the web site. Bob volunteered to develop a contact list for the Academy. This list will serve as campus contacts for e-mails who can then forward to members on their respective campuses. In addition, Bob has graciously volunteered to serve as the Public Relations member of the Executive Council.

Fellows: Krisma DeWitt reported no new nominations for Fellows for 2007. Krisma graciously agreed to head the 2008 Fellows Committee. Nominations for Fellows for 2008 will be sent to Krisma.

Membership: Bob Tatina will deliver the membership report at the business meeting.

Resolutions: Gary Larson generously volunteered to head this committee

Nominations: Jim Sorenson will head the nominations committee. Positions to be filled are second VP and members-at-large.

The following resolution will be presented to the membership at the Business Meeting. Jim suggested that a commendation for Bob Tatina for the 2006 resolution he authored and forwarded to the South Dakota Board of Education on inclusion in the new set of administrative rules to include statements on the molecular basis of education and the scientific theory and biological principles of evolution.

Jim Sorenson reported on the continuation of an opportunity through the AAAS to provide awards to students. For the undergraduate award, to be pre-
sented to one male and one female undergraduate student. Dave Bergmann will head this committee, assisted by Krisma DeWitt and Donna Hazelwood.

Jim has requested from Bob Tatina for the fall executive council meeting, a manual for duties of the President.

Kristel Bakker and Donna Hazelwood sent eight award checks for $25.00 each to five South Dakota Regional Science Fairs for a total of 40 checks and $1000.00. The regional science fair coordinators are: 1) Jodie Ramsay, Northern South Dakota Science and Math Fair; 2) Madeline Rose, Eastern South Dakota Science and Engineering Fair; 3) Brian T. Hemmelman, High Plains Regional Science and Engineering Fair; 4) Michael Nobel Farney, South Central South Dakota Science and Engineering Fair; and 5) Monica Mayer, Northwest Area Schools Regional Science and Engineering Fair. The SDAS presence at the Regional Science Fairs was discussed. Four regional Science Fairs applied for continuing support by the Academy for 2008.

Nels Troelstrup suggested that an item for the proposed education section of the Academy that the winners of the regional science fairs be invited to present at the annual meeting of the Academy. Krisma DeWitt is spearheading the effort to create an education section.

Jim Sorenson proposed that the fall 2007 executive council meeting be held Saturday 22 Sept. 2007 at Cedar Shore Resort. He also suggested that formal invitations on SADS letterhead stationery be used to send invitations to present and attend the academy. In addition, he proposed adding a signal transduction paper session for the 2008 meeting.

Jim proposed that to increase representation on the Executive Council, the number of members-at-large be increased from four to six. This would involve electing one new member-at-large for a one year term and two members-at-large for two year terms.

Jim Sorenson opened the business meeting Saturday 14 April 2007 and offered a thank-you for the accommodations to Nels Troelstrup Jr. and the other members of the host committee.

Incoming President Mike Wanous gave a well crafted, stimulating and thought provoking Presidential Address “Evolution and Faith: Complimentary or Conflicting Visions?”

Registration for the Symposium and Annual Meeting began 10:00 a.m. Friday 13 April, continued again 8:00 a.m. Saturday 14 April. Steve Chipps brought Terri Symens to again assist with registration. Carol Jacobson and Diane Drake also assisted.

The Symposium on “Signal Transduction” was organized and hosted by Mike Wanous and included ten presentations. The event was highlighted by the Symposium Keynote Address by Dr. Kathleen Eyster, Sanford School of Medicine, USD, on “New Paradigms in Signal Transduction.”

The Plenary Speaker, Dr. Thomas Lovejoy, President of the H. John Heinz III Center for Science, Economics, and the Environment” gave a thoughtful and timely presentation on the “Global Climate Change and Biodiversity.”

The Treasurer report was provided by Donna Hazelwood for Kristel Bakker. Audrey Gabel and Mike Gabel served as Auditing Committee. Audrey reported
that the account was in order. Mike Wanous moved and Mark Gable seconded that the treasurer’s report be accepted. The motion passed by voice vote. The CD at Dakotah Bank will be allowed to rollover for another term.

The Resolutions committee, proposed the following resolutions: 1) thanks to South Dakota State University for hosting the event and to Nels Troelstrup, Jr. and the local planning committee Jim Sorenson, Steve Chipps, Donna Hazelwood, and Terri Symens; 2) thank you to the Symposium Committee Mike Wanous and Jim for arranging the Signal Transduction Symposium and Keynote Speaker; 3) thank you to Keynote Speaker Dr. Kathleen Eyster, USD Sanford School of Medicine, who spoke on “New Paradigms in Signal Transduction”; 4) thank you to Nels Troelstrup Jr. for arranging the Plenary Speaker; 5) thank you to Plenary Speaker, Dr. Thomas Lovejoy, President of the H. John Heinz III Center for Science, Economics, and the Environment, who spoke on “Global Climate Change and Biodiversity”; 6) commend Past President Jim Sorenson for his direction and leadership 2006-2007, 5) thanks to Terri Symens, Di Drake, and Nancy Presuhn for secretarial assistance and to Terri Symens for assisting with registration for the meeting, 6) thanks to Mike Wanous for presidential address on “Evolution and Faith: Complimentary or Conflicting Visions?”; 7) thanks you to Terri Symens for assisting with registration at the meeting; 8) thanks to Secretary Donna Hazelwood and Treasurer Kristel Bakker for continued service, and 9) a special thanks goes to Editor Steve Chipps for his oversight of timely publication of the Proceedings. Steve Chipps moved and Mike Wanous seconded acceptance of the resolutions. The motion carried by voice vote.

Jim Sorenson read a resolution commending Bob Tatina for the 2006 resolution he authored and forwarded to the South Dakota Board of Education on inclusion in the new set of administrative rules to include statements on the molecular basis of education and the scientific theory and biological principles of evolution. Donna Hazelwood moved and Gary Larson seconded acceptance of the resolution. The resolution carried by voice vote.

Bob Stoner suggested that a copy of the Presidential Address given by Mike Wanous be forwarded to the SD Board of Education by Bob Tatina.

Bob Tatina reported that of the 121 members in attendance, 62 were new members. He moved and Neil Reese seconded acceptance of the 62 new members. The motion carried by voice vote.

On behalf of the Executive Council, Jim brought forth a proposal to add the Webmaster to the Executive Council. Neil Reese is the current Webmaster. Donna Hazelwood moved and Krisma DeWitt seconded acceptance. The proposal carried by voice vote. Maureen Diggins suggested that information from the member institutions on events of interest be forwarded to the webmaster for inclusion on the Academy web page.

Jim forwarded a proposal from the Executive Council to increase the number of Members-at-Large from four to six. The rationale is to increase the representation from institutions in the state. Jim moved and Bob seconded acceptance. The motion passed by voice vote.

The results from the AAAS undergraduate student award were announced by the selection committee chair Dave Bergmann. Dave stated that the selection
process was difficult and that the committee, Krisma DeWitt and Donna Hazelwood, suggested including two honorable mention posters for each category. In addition, he suggested to keep the background simple and use bold font.

Male winner

Ellis, Kevin (UG) and Deig N. Sandoval. 2007. Hypoglycemic Effects of *Momordica charantia* in Diabetic Animal Models. OLC-Lakota Institute for Science and Technology. (poster 47)

Female winner

Magee, Christine A. (UG), Alicia A. Goyeneche, Grigory A. Sereda, and Carlos M. Telleria. 2007. Newly Synthesized Bicyclic Quinones as Potential Anti-ovarian Cancer Agents. USD-Division of basic Biomedical Sciences and Chemistry. (poster 21)

Male Honorable mention (alphabetical order)

Henriksen, Cody (UG), Mandi Greenway (UG), Gina Furman, John Brannan, Kathleen Eyster, and Maureen Diggins. Augustana College and Sanford School of Medicine. (poster PO-07)

Davidson, Anders J. (UG), Alex C. Johnson (UG), Katie L. Severson (UG), and Jetty L. Duffy-Matzner. Augustana College. (poster PO-17)

Female Honorable mention (alphabetical order)

Eslinger, Allison P (UG)., Lindsey D. Rieck, Mindy Jo Knudson, and Kristi A. Egland. 2007 Characterization of CAPC in Multiple Cancers. Augustana College and Signal Transduction Institute-SD Health Research Foundation-Sanford School of Medicine. (poster PO-05)


Kevin Ellis and Christina Magee will each receive a one year membership in the AAAS.

Steve moved and Mike Wanous seconded honoraria for Terri, Di, and Nancy in the amounts of $600.00, $100.00, and $100.00 respectively.

Elections were held for officers for 2007-2008. The nominations committee, Jim Sorenson and Bob Tatina presented the nominations. Nels moved and Maureen Diggins seconded nominations cease and members cast a unanimous ballot in favor of Second Vice President; Dave Bergmann from BHSU; for 2007-2008 Member-at-Large; Jeffrey Palmer from DSU; and 2007-2009 Members-at-large; Mike Barnes from SDGF&P, Gary Larson from SDSU, and Chun Wu from Mount Marty.

Krisma Dewitt has graciously agreed to continue as Chair of the Fellows Committee.

Bob has kindly agreed to continue as Chair of the Publicity Committee and will be sending out information on the need to recruit new members.
On behalf of the membership committee, Nels Troelstrup recommended that the Academy accept as new members 120 individuals. Bob moved and Jim Lefferts seconded the motion. The motion carried by voice vote.

Next year Mount Marty will host the 93rd annual meeting 4-5 April at Cedar Shore. Following similar format, a keynote speaker will address the Academy and we will invite a symposium. Anyone interested in participating in the symposium is invited to visit with Jim, Steve, or Donna.

Steve requested that the Academy provide honoraria for assistance in the following amounts to Terri Symens $500.00, Di Drake $100.00, and Nancy Presuhn $100.00. Neil moved and Donna seconded a motion to give the amounts requested. The motion carried by a voice vote.

Committee positions for 2007-2008 include

- Membership: to be filled
- Fellows: Krisma DeWitt
- Resolutions: to be filled
- Nominations: to be filled
- Publicity: Bob Tatina

Outgoing President Jim Sorenson handed the hammer of office to Incoming President Mike Wanous. First Past President Bob Tatina presented outgoing President, Jim Sorenson, a plaque honoring his contributions to the Academy.

Steve Chipps moved and Mike Wanous seconded the meeting be adjourned. The motion carried by voice vote.

Several items for consideration at the fall meeting of the Executive Committee were discussed. 1) the 2007 meeting hosted by SDSU; 2) nomination of individuals for Fellow; 3) recruitment of new members; 4) the 2008 meeting hosted by Mount Marty College; 5) the locations of future meetings, 6) exposure and publicity of the academy, and 7) forwarding resolutions to Pierre.

To Recap: the SDAS 2006 Annual Meeting hosted by Dakota Wesleyan University included of a Symposium on “Energy Resources in South Dakota: Issues and Global Concerns” hosted by Perry Rahn and included eight presentations. Saturday, 48 posters and 24 papers were presented. A total of 89 members attended the 2006 Annual Meeting.

Respectfully submitted,
Donna Hazelwood, DSU
SDAS Secretary

REPORT OF THE RESOLUTIONS COMMITTEE

The South Dakota Academy of Science is grateful to Jim Sorenson for his wise and capable leadership over the past year. We also thank the local planning committee, headed by Nels Troelstrup and assisted by Matt Miller, Jerome Krueger, Scott Pedersen, Sharon Clay and Gary Larson, for organizing the 2007
meeting. We thank David Chicoine, President of SDSU, for his kind words of welcome to the membership.

Congratulations and thanks to Michael Wanous for organizing a highly successful symposium on “Signal Transduction”.

We much appreciate the secretarial help provided by Terri Symens and thanks to both Terri and Carol Jacobson for their help at the registration desk.

We are grateful to Thomas Lovejoy for his keynote address on global climate change and his sobering message about present and future impacts on biological diversity and human populations. We also recognize and thank the F.O. Butler Foundation and the following individuals for helping to finance Dr. Lovejoy’s visit to SDSU: Gary Lemme, Dean of the College of Agriculture and Biological Sciences, John Kirby, Assoc. Dean and Director of the Experiment Station, Don Marshall, Assoc. Dean and Director of Academic Programs, and Thomas Cheesbrough, Head of the Department of Biology & Microbiology.

Finally, the Academy wishes to thank Steve Chipps, Editor of the Proceedings, Donna Hazelwood, Secretary, Kristel Bakker, Treasurer, and Neil Reese, website designer, for their continued service to the Academy.

Respectfully submitted,
Gary Larson, Resolutions Committee

SOUTH DAKOTA ACADEMY OF SCIENCE
2007–2008 EXECUTIVE COUNCIL

President        Michael Wanous, Augustana College, Biology
                 274-4712, mike_wanous@augie.edu
President-Elect  Nels H. Troelstrup, Jr. SDSU, Biology and Microbiology
                 688-5503, Nels.Troelstrup@sdstate.edu
First Vice-President        Krisma DeWitt, MMC Chemistry
                           668-1530, kdewitt@mtmc.edu
Second Vice-President    Dave Bergmann, BHSU, Biology
                          652-2420, DaveBergmann@bhsu.edu
Secretary              Donna Hazelwood, DSU, Biology
                        256-5187, Donna.Hazelwood@dsu.edu
Treasurer              Kristel Bakker, DSU, Biology
                        256-5182, Kristel.Bakker@dsu.edu
Proceedings Editor     Steve Chipps, SDSU, Wildlife and Fisheries, USGS,
                      688-5467, Steven.Chipps@sdstate.edu
First Past President   James Sorenson, MMC, Biology
                      668-1581, jsorenson@mtmc.edu
Second Past President  Robert Tatina, DWU, Biology
                      995-2712, rotatina@dwu.edu
Publicity             Andrew Detwiler, SDSM&T, IAS
                      394-1995, Andrew.Detwiler@sdsmt.edu
Publicity             James Sorenson, MMC, Biology
                      668-1581, jsorenson@mtmc.edu
Publicity  Robert Tatina, DWU, Biology
995-2712, rotatina@dwu.edu
Webmaster  Neil Reece, SDSU, Biology
688-4568, neil.reece@sdstate.edu

Members-at-Large
2006-2008  John Naughten, NSU, Biology
626-2456, naughtej@northern.edu
2006-2008  David Swanson, USD, Biology
677-6175, dlswanso@usd.edu
2007-2008  Jeffrey Palmer, DSU, Mathematics
256-5190, Jeffrey.Palmer@dsu.edu
2007-2009  Mike Barnes, SDGFP
642-6920, mike.barnes@state.sd.us
2007-2009  Gary Larson, SDSU, Biology/Microbiology
688-6141, gary.larson@sdstate.edu
2007-2009  Chun Wu, MMC, Chemistry
668-1381, cwu@mtmc.edu

SOUTH DAKOTA ACADEMY OF SCIENCE
TREASURER’S REPORT 13-APR-07

Balance as of 14-October-05  $9962.68

Debits 2006:
2006 SDAS meeting:
Ted Patzek 500.00
Terri Symens (room) 49.52
SDSMT 36.00
Printing 21.00
Di, Nancy, Terri (thank yous) 700.00
Cedar Shores 3045.77
U-Haul (Miles K) 77.78
Science Fair 1000.00
US Post Office 16.40
Total $5446.47

Credits:
2006 SDAS meeting $4695.00

Balance as of 14-October-2006 $9211.21

Debits 2007:
Cedar Shores (Fall Executive Board Meeting) 93.38
Science Fair 2007 1000.00

Balance as of 13-April-2007 $8150.73

Respectfully,
Kristel K. Bakker
SDAS Treasurer
SOUTH DAKOTA ACADEMY OF SCIENCE
APRIL 13, 2007—PREPARED BY DI DRAKE
PROCEEDINGS DISBURSEMENTS / RECEIVABLES

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Cash Balance in SDSU-SD Academy of Science Proceedings Account

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SDAS 2007 AAAS UNDERGRADUATE POSTER COMPETITION AWARDS

Names of winners are in **bold**.

**Male Winner**

Ellis, Kevin and Deig N. Sandoval. 2007. Hypoglycemic Effects of *Momordica charantia* in Diabetic Animal Models. OLC-Lakota Institute for Science and Technology. (poster 47) kellis10489@mail.olc.edu

**Female Winner**

Magee, Christine A., Alicia A. Goyeneche, Grigory A. Sereda, and Carlos M. Telleria. 2007. Newly Synthesized Bicyclic Quinones as Potential Anti-ovar-
ian Cancer Agents. USD-Division of basic Biomedical Sciences and Chemistry. (poster 21) cmagee@mtmc.edu

Male Honorable Mention

Henriksen, Cody, Mandi Greenway, Gina Furman, John Brannian, Kathleen Eyster, and Maureen Diggins. Augustana College and Sanford School of Medicine. (poster PO-O7). no e-mail listed on registration form.

Davidson, Anders J., Alex C. Johnson, Katie L. Severson, and Jetty L. Duffy-Matzner. Augustana College (poster PO-17) no e-mail listed on registration form.

Female Honorable Mention

Eslinger, Allison P., Lindsey D. Rieck, Mindy Jo Knudson, and Kristi A. Egland. 2007 Characterization of CAPC in Multiple Cancers. Augustana College and Signal Transduction Institute-SD Health Research Foundation-Sanford School of Medicine. (poster PO-O5) no e-mail listed on registration form.

Weyrich, Laurie, Y. Luo, J. Sutton, S. Vilan, and V. Brözel. 2007. Genes Contributing to the Induced Multicellularity of Bacillus cereus Growing in Soil. SDSU. Biology/Microbiology. (poster 52) lsweyrich@jacks.sdstate.edu
When I was considering what I should talk about this afternoon, I wanted to choose a topic where I could make a small, but hopefully significant, contribution. As a Christian, geneticist, and professor of biology, I have thought a lot about this topic, “Evolution and Faith”. After settling on this title, I thought to myself, “Why pick such a controversial topic?” But then I remembered the recent article in Science magazine reporting that significantly less than half of adults in the United States accept the idea of evolution, and that the number is declining. And this is in the face of amazingly compelling evidence for evolution, coming from multiple directions, including the Human Genome Project. While South Dakota is too far north to be considered part of the “Bible belt”, if you have ever taught evolution to a class of South Dakota college students, or read the Argus Leader recently, you know that we fit the trend of rejecting the Theory of Evolution pretty well. In fact, we are probably close to the leading edge. I would say that South Dakota is the “Bible necklace”. So, what better place to have this conversation than the South Dakota Academy of Science?

Less than a year ago, Jon Miller, Eugenie Scott, and Shinji Okamoto published their study of the public acceptance of evolution in Science magazine. They compared surveys taken in 34 countries over several years. American adults were asked whether the statement, “Human beings, as we know them, developed from earlier species of animals”, is true, false, or whether the respondent is not sure or does not know. In 1985, 45% of Americans considered this statement true, 7% weren’t sure, and 48% considered the statement false. In 2005, the responses were 40% true, 21% not sure, and 39% false. So, over 20 years, those accepting human evolution declined from 45 to 40%, those rejecting the idea declined from 48 to 39%, and those not sure increased from 7 to 21%. Americans accepting and rejecting human evolution are now about evenly split, with 21% unsure. Digging deeper, Miller et al., reported that in 1993 and 2003, Americans were asked a similar question, but were given more response choices: “definitely true, probably true, probably false, definitely false”, or not sure or don’t know. About a third thought human evolution was definitely false, 14% thought human evolution was definitely true, and combining the other responses, about 55% had varying levels of uncertainty about evolution.
It is interesting to speculate on why the numbers sure of their answers have declined on both sides, while those unsure have increased dramatically. In the last 20 years, proponents of creationism, and more recently Intelligent Design, have been very actively fighting the idea of evolution. Concurrently, the evidence for evolution has improved, with key transitional forms in the fossil record being described, and DNA sequence analysis providing compelling evidence for common descent. So, maybe Americans are just confused by the conflicting stories? A more sobering possibility is that Americans don’t have the science background to form an educated opinion (as was well documented by Bob Tatina in his presidential address two years ago), or in our frantic and entertainment-saturated culture, we don’t take the time to think about such an important issue, or don’t care.

Looking at the simpler question about human evolution with just three answers, they also found that among people surveyed in 34 countries, including Europe, Japan, and the United States, the U.S. had the lowest level of public acceptance of evolution except for Turkey. They gave this explanation:

“the structure and beliefs of American fundamentalism historically differ from those of mainstream Protestantism in both the United States and Europe. The biblical literalist focus of fundamentalism in the United States sees Genesis as a true and accurate account of the creation of human life that supersedes any scientific finding or interpretation. In contrast, mainstream Protestant faiths in Europe (and their U.S. counterparts) have viewed Genesis as metaphorical and—like the Catholic Church—have not seen a major contradiction between their faith and the work of Darwin and other scientists.”

To test their hypothesis that religion was having an effect on Americans’ view of evolution, they created a model to predict attitude toward evolution utilizing 10 independent variables, including religious belief. They found that the effect of fundamentalist religious belief was nearly twice as high in Americans as in Europeans, and that those holding “a strong belief in a personal God and who pray frequently” were significantly less likely to believe in evolution.

So, is it possible for someone who holds a strong belief in a personal God to also believe in evolution? This is an important question for the community of faith, as well as the scientific community. I would hope that all people of faith would want to possess as complete a worldview as possible, one that accurately reflects what we know about the world. I would also hope that those charged with educating students about evolution would want to understand how to most effectively communicate this concept, even if they are not themselves believers in God. As a member of both communities, I would like to address both in the hope of increasing understanding, as well as encouraging productive dialogue on the topic of evolution and faith.

For the community of faith, I would like to summarize why I believe evolution to be the best explanation for our observations of life on this planet, both past and present. There are a number of independent lines of evidence, which
converge to form a very compelling case for evolution. First, let us consider the power of selection on organisms. Natural selection is the selection, or survival and reproduction, of adapted individuals in nature. But there are examples of human selection that we see every day. The cole crops cauliﬂower, cabbage, Brussels sprouts, broccoli, kale, and kohlrabi are all the same species of plant, which have been selected by humans to emphasize different traits. The numerous breeds of dogs are another example of how selection (by humans) can push organisms in very different directions in the short span of a few hundred years.

Second, biogeography points to evolution. An example of this is life on islands, where species have been found to radiate into different types to exploit different niches. When these very different looking species are closely examined, they are found to be related to one mainland species. The greatest example of this is perhaps seen in Australia, where numerous marsupial forms of mammals are found.

Third, the fossil record shows a clear progression of increasing complexity in life over time on this planet. The deeper (older) layers of fossils contain simpler organisms, and complexity increases with less deep (younger) fossils. For example, the order of appearance is bacteria, invertebrates, ﬁsh, amphibians, reptiles, and ﬁnally mammals and birds. While there is not a complete record of all evolutionary transitions in the fossils, the trend is clear. When we consider the conditions necessary for fossil formation, it is not surprising that there should be gaps in what was preserved. In recent years, key transitional forms have been discovered, such as mammal-like reptiles, and whale intermediates. Creationists hold that the radioactive dating methods used to date fossils are not accurate. In fact, the assumptions made are reasonable, and radioactive decay is known to occur at exact and reproducible rates. For example, phosphorus-32 is known to have a half-life of 14.3 days. You can measure this in the lab time after time and you will observe that the rate of decay is constant. The estimated age of the earth is 4.5 billion years. Even if the assumptions of radioactive dating were off, and let’s say we got an age of 1 billion years, we would still be talking about orders of magnitude between this age and 6,000-10,000 years, the age held to by creationists. Clearly, these two views are irreconcilable.

Fourth, in the ﬁeld of comparative anatomy, very different mammals are found to have homologous structures. For example, if you examine the forelimbs of humans, cats, whales, and bats, you will ﬁnd that although these limbs vary greatly in shape and function, they share a common set of bones. This is most easily explained by common ancestry with the different descendants being molded by different selective pressures to suit different conditions. Vestigial structures also point to modiﬁcations of ancestral structures over time. Examples include pelvic bones in snakes and cave animals with blind eyes. These creatures have no use for these structures; rather they appear to be “leftovers” from ancestors, which are no longer used and are in the process of being lost.

Fifth, perhaps the greatest evidence for common ancestry among organisms comes from the comparison of gene sequences between species. When we compare the DNA (or the encoded protein) sequences between different organisms, we ﬁnd that the more closely related according to evolutionary expectations two
species are, the more similar are their DNA sequences. For example, in comparing the human hemoglobin protein sequence with other animals, the rhesus monkey shows 95% identity, the mouse 87%, the chicken 69%, the frog 54%, and the lamprey 14% identity with the human sequence. This is exactly what would be expected if these species shared a common ancestor with humans, and there had been increasing amounts of time from the point of a shared ancestor. If we look at how the genes are organized in the chromosome, we again see evidence of evolution. Among species in the same taxonomic group, there are long stretches of conserved gene order (colinearity) on chromosomes. This is very striking at the Family level, but is also seen at the Class and Phylum levels. For example, in comparing human and cat chromosomes, cat chromosomes contain very long stretches of genes that line up with the gene order of human chromosomes. In fact, most cat chromosomes are composed of stretches of genes that correspond to only one, two, or three human chromosomes. This is the pattern that would be expected if chromosomes from a common ancestor were slowly rearranged over time as new species evolved.

Francis Collins, a Christian, physician, geneticist, and Director of the Human Genome Project, recently published a book, *The Language of God*, which is the best book I have read on the relationship of faith and evolution. In his book, Collins points out that not only is the order of genes conserved between related species, but the non-functional repeated sequences between genes also show a pattern of conservation. For example, ancient repetitive elements (AREs) have built up in many genomes as these “jumping genes” have multiplied and repeatedly re-inserted themselves in genomes over time. Comparing the mouse and human genomes, stretches of conserved AREs are also found between the stretches of gene colinearity. In fact, copies that were damaged during the transposition process can be found in the same relative positions in the mouse and human genomes. It is hard to imagine why a Designer would insert damaged genes in exactly the same positions in the genomes of similar species. Collins also points out that humans and chimpanzees share 96% identity at the DNA level, and that our chromosomes are the same except for two chimp chromosomes that appear to have fused end-to-end to form a single chromosome in humans. The patterns of genes, non-functional DNA, and chromosomes all point strongly to a process starting with common ancestry showing genome rearrangement over time as species diverge from each other.

So, what is a Christian to make of all this evidence? I reject the belief held by some creationists that God put the evidence there to test our faith. This conception of the character of God is not consistent with what I know about him. The most straightforward interpretation is that God used an evolutionary process in his work of creation. As Galileo said, “I do not feel obliged to believe that the same God who endowed us with sense, reason, and intellect, had intended for us to forgo their use.” We should be careful about interpreting the Genesis creation account as a scientific document and using this interpretation as our lens to understand the natural world. This is what the Church did with Galileo when it punished him for teaching that the earth revolved around the sun, and not vice versa. I contend that it is quite possible to believe in the Creator and Genesis without being a creationist! We should take into account the literary genre of
the Genesis creation narratives. They do not appear to have been intended as a scientific description of how God created, but rather tell us about who God is as creator, and who we are in relation to him. The majority of conservative biblical scholars agree that there is a significant element of symbolism in the Genesis creation accounts. Roy Clouser, in his excellent article, *Genesis on the Origin of the Human Race*, warns against the danger of using the Bible as an encyclopedia in order to find shortcuts to knowledge about the natural world. He states, “The ‘encyclopedic assumption’ ignores the Bible’s own central theme and purpose, and tries to force the text to yield truths about matters which never crossed the minds of its authors.” Many scientists who are also Christians subscribe to the idea going back to St. Augustine that God has given us two books of revelation: the book of scripture and the book of nature. If we ever get to the point where we understand both of these books perfectly, there will be no contradiction between them.

Creationism poses several threats to the community of faith. First, it forces young people to choose between faith and scientific evidence. Some, having been told that they have to make this choice, abandon their faith. Thus, those who think they are protecting the faith are actually driving the faithful away. Second, as Paul Rohde, campus pastor of Augustana College shared with me, the converse danger is that “faithful people would abandon their minds, senses, and critical capacity”. Another danger of creationism (and Intelligent Design) is to the credibility of faith. A “God of the gaps” theology squeezes God’s role in creation into the parts that we do not yet understand. But as scientific knowledge expands, God is relegated to a smaller and smaller place. Collins quotes from St. Augustine in 400 AD, more than a millennium before Darwin:

“In matters that are obscure and far beyond our vision, even in such as we may find treated in Holy Scripture, different interpretations are sometimes possible without prejudice to the faith we have received. In such a case, we should not rush in headlong and so firmly take our stand on one side that, if further progress in the search of truth justly undermines this position, we too fall with it.”

Now I would like to turn my attention to the scientific community. It is the zealots on both extremes who are doing the most damage. A prime example is Richard Dawkins, who in his recent bestseller, *The God Delusion*, states “I am not attacking any particular version of God or gods. I am attacking God, all gods, anything and everything supernatural, wherever and whenever they have been or will be invented”. In a debate between Dawkins and Collins, published by *Time* magazine in November, 2006, Dawkins stated: “Once you buy into the position of faith, then suddenly you find yourself losing all your natural skepticism and your scientific—really scientific—credibility. I’m sorry to be so blunt.” Dawkins extends the methodological naturalism that all scientists use in their work to a philosophical naturalism that pervades his whole outlook. There is no room for any belief in the supernatural. It is seen as a result of a weak mind, as a delusion, something dangerous to be eradicated from the world.
Scientists who use science or evolution as a weapon in their crusade for atheism not only overstep the boundaries of what science can answer, but also make the dialogue with the public much more difficult. As Lawrence Krauss pointed out recently in New Scientist, “Not only is it inappropriate to try to convince people of the validity of scientific theories by first arguing that their deeply held beliefs are silly, it is also clear that the existence of God is a metaphysical question which is, for the most part, outside the domain of science. Now more than ever it is important to understand the limits of science.”

Stephen Jay Gould, one of the most articulate explainers of evolution, placed science and religion in what he called “non-overlapping magisteria”. He wrote,

“To say it for all my colleagues and for the umpteenth millionth time… science simply cannot (by its legitimate methods) adjudicate the issue of God’s possible superintendence of nature. We neither affirm nor deny it; we simply can’t comment on it as scientists.”

Gould had little patience for either science or religion infringing on the other’s turf. He continued,

“If some of our crowd have made untoward statements claiming that Darwinism disproves God, then I will find Mrs. McInerney [Gould’s third-grade teacher] and have their knuckles rapped for it (as long as she can equally treat those members of our crowd who have argued that Darwinism must be God’s method of action). Science can work only with naturalistic explanations; it can neither affirm nor deny other types of actors (like God) in other spheres (the moral realm, for example).”

Gould then went on to demonstrate that prominent proponents of evolutionary theory have included both agnostics and devout Christians. He concluded that evolutionary theory is compatible with both conventional religion and atheism. My only difference with Gould here is that I would add that the two spheres are complementary, synergistic, not merely non-overlapping. Returning to the thoughts of Lawrence Krauss, aggressive atheism “pitch[es] misguided evangelicals against the scientific community….To counter these threats we need to argue compellingly that people of faith are ill served by ignorance, rather than argue that faith and ignorance are synonymous.”

To many observers, the beauty, order, and magnificence of nature point to the Creator. They declare his power and divine nature. As the scriptures say in Psalm 19, “The heavens proclaim the glory of God. The skies display his craftsmanship.” Let’s take the Big Bang as an example. Science can never tell us the Why behind the Big Bang, and so far has not been able to tell us the How. But Genesis 1:3 states “And God said, ‘Let there be light…” This is the Who, and the rest of the story is the Why. The message of faith is that God’s revelation in scripture provides us with answers to questions that science cannot address, such as “What is our purpose?” “Who is God?” “Who are we?” If nature points us to God’s ordered mind, the scriptures tell us about his heart. The scriptures record
the history of God progressively revealing himself to us over time. This is a type of truth different from what science can provide.

To summarize, if believers could see the two books of God’s revelation as complementary rather than conflicting, and if scientists were clearer on the point that science does not, cannot, disprove God, we could start seeing less conflict and confusion and more of an appreciation that these two viewpoints complement each other to give us a more complete picture of the whole of reality. I will conclude with a final thought from Galileo: “scripture teaches us how to go to heaven, [and science] how the heavens go”.

RESOURCES


HABITAT USE AND MOVEMENTS OF ADULT PALLID STURGEON IN THE MISSOURI RIVER DOWNSTREAM OF FORT RANDALL DAM, SOUTH DAKOTA AND NEBRASKA

Greg A. Wanner, Robert A. Klumb and Wayne J. Stancill
United States Fish and Wildlife Service
Pierre, SD 57501

George R. Jordan
United States Fish and Wildlife Service
Billings, MT 59101

ABSTRACT

Ultrasonic telemetry was used from 2000 to 2002 to identify habitat use and track seasonal and diel movements of six adult pallid sturgeon (Scaphirhynchus albus) released in the Missouri River downstream of Fort Randall Dam, South Dakota and Nebraska. Extensive sampling occurred at about two week intervals from spring through fall. Two individual fish were intensively tracked for 4 to 12 hours during 2000 to assess diel movements, with one individual tracked on three occasions. A total of 29 relocations were observed from four pallid sturgeon and two fish were only found once after the initial year of stocking suggesting a survival rate of 33%. In all seasons, adult pallid sturgeon were located in the main river channel habitat and at relative depths ranging from 79 to 100% of the maximum channel depth. During the multiple year study, two different movement patterns were observed of the two fish. One of the fish moved throughout the study area while the other fish moved downstream below the Missouri and Niobrara rivers confluence and remained there throughout the study period. In general, both fish moved upstream during late fall through the spring and moved downstream during the summer. Both fish also were relocated in two to three distinct areas of the Missouri River, a potential indication of preference to unknown biotic and abiotic habitat conditions. One fish had a maximum observed range of 8.1 km with low seasonal movement rates compared to the other fish that had a maximum observed range of 45.8 km. The pallid sturgeon that was intensively tracked on three occasions had substantially higher (≥ 40%) movement rates at night compared to dawn, daytime, and dusk. Although the number of tagged fish in this study was small, all field observations for an endangered species are valuable for recovery efforts. Observations from the two fish in this study were consistent with other studies which showed that adult pallid sturgeon are a highly mobile, wide-ranging species.
INTRODUCTION

Pallid sturgeon (*Scaphirhynchus albus*), a species native to the Missouri River system (Forbes and Richardson 1905), was listed by the U. S. Fish and Wildlife Service (USFWS) as an endangered species in the fall of 1990 (USFWS 1990). Conversion of the free flowing upper Missouri River into a series of impoundments and extensive channelization of the lower Missouri River has resulted in habitat degradation and an altered hydrograph that are suspected for the decline in distribution and abundance of pallid sturgeon (Kallemeyn 1983). Pallid sturgeon are benthic and tend to occupy main channel habitats in the lower Mississippi River (Hurley et al. 2004) and main channel areas associated with islands or sand bars in the upper Missouri River (Bramblett and White 2001). Because all rivers within the historic range of pallid sturgeon are highly modified (Kallemeyn 1983), habitats occupied by the fish likely are not necessarily the species’ “preferred” habitat.

An inter-reservoir reach of the Missouri River downstream of Fort Randall Dam, South Dakota (Figure 1) has been listed as one of six recovery priority management areas (RPMA) for pallid sturgeon based on remnant riverine habitat characteristics at the time of listing (USFWS 1993). As part of the recovery efforts for pallid sturgeon, the USFWS initiated a stocking program in RPMA 3 in 2000 and released 416 age-3 hatchery-reared juvenile pallid sturgeon in this reach of the Missouri River. An additional three adult pallid sturgeon used as brood stock were also released with the juveniles.

Biotelemetry studies provide insight on fish movement and habitat use. No telemetry studies using adult pallid sturgeon have been published for the unchannelized Missouri River downstream of Fort Randall Dam. Previous telemetry studies in the Missouri River focused on the unchannelized river in Montana and North Dakota (Bramblett and White 2001) and on a main stem South Dakota reservoir, Lake Sharpe (Erickson 1992). Movements and habitat use of adult pallid sturgeon have also been studied in the middle Mississippi River (Hurley 1999; Hurley et al. 2004).

Recovery of pallid sturgeon and assessment of RPMA 3 as a recovery area necessitates studies encompassing all life stages. Jordan et al. (2006) reported that hatchery-reared juvenile pallid sturgeon used the entire reach of RPMA 3 and had a high survival rate (> 65%). However, it is not known whether this inter-reservoir reach of the Missouri River will support an adult pallid sturgeon population. A self-sustaining population is the ultimate determinate of recovery success identified in the pallid sturgeon recovery plan (USFWS 1993). Thus, the objectives of this study were to document seasonal and diel movement patterns and general habitat use of adult pallid sturgeon in this inter-reservoir reach of the Missouri River (RPMA 3).
STUDY AREA

Gavins Point Dam on the Missouri River was closed in 1955 which formed Lewis and Clark Lake (Figure 1). This reservoir forms a boundary between the states of South Dakota and Nebraska and can be subdivided into two distinct habitats. The upper reach extends for approximately 76 river km (rkm) from Fort Randall Dam (rkm 1,416) to near Springfield, South Dakota (rkm 1,340) and still retains many natural riverine characteristics including: sand bars, old growth riparian forest, side channels, and year round flows. Because of these remaining riverine characteristics, this upper reach was chosen for stocking juvenile hatchery-reared pallid sturgeon as part of the recovery plan (USFWS 1993; Jordan 2006). However, discharge is regulated by Fort Randall Dam resulting in unnatural daily fluctuations (> 0.75 m) and seasonal discharge patterns. The second habitat, downstream of Springfield, South Dakota to Gavins Point Dam (rkm 1,305), is comprised of the reservoir, Lewis and Clark Lake.

METHODS

Six adult pallid sturgeon (mean fork length [FL] = 1,321 mm [SE = 55] and mean weight = 15.1 kg [SE = 1.9]) were surgically implanted with sonic transmitters (Sonotronics, Tuscon, Arizona) on 2 June 2000. These six fish were captured in Lake Sharpe, South Dakota in 1992 and maintained for eight years at Gavins Point Dam National Fish Hatchery (NFH) in Yankton, South Dakota. Water temperatures at the hatchery at the time of tagging were

Figure 1. Map showing the Missouri River downstream of Fort Randall Dam where four sonic-tagged adult pallid sturgeon were released near Verdel, Nebraska on 6 July 2000 (star) and where two adult pallid sturgeon were released near Running Water, South Dakota on 20 September 2000 (diamond) and tracked from 2000 to 2002. The Missouri River was divided into four zones for randomization of tracking effort.
No anesthesia was used during surgery and the fish were held after surgery in the hatchery for five weeks to assess tag expulsion and mortality. Antibiotics were administered following surgery to reduce bacterial infection. Following this holding period, the surviving fish were released into the Missouri River near Verdel, Nebraska (rkm 1,377) on 6 July 2000. Another two adult pallid sturgeon (mean FL = 1,403 mm [SE = 33] and mean weight 15.4 kg [SE = 5.7]) had sonic transmitters surgically implanted on 15 August 2000 and were released in the Missouri River near Running Water, South Dakota (rkm 1,360) on 20 September 2000. These two fish were originally captured in the Missouri River downstream of the Yellowstone River Confluence in North Dakota in 1997 and maintained at Gavins Point NFH for three years.

Transmitters were 60 mm in length and 18 mm in diameter. Transmitter dry weight was 25 g and the transmitter weight to fish body weight ratio ranged from 0.1 to 0.3%. Each transmitter emitted a unique aural code which identified an individual fish and had a life expectancy (battery life) of 36 months. Frequencies emitted by the sonic tags ranged from 71 to 78 kHz. The six adult pallid sturgeon in this study were relocated in conjunction with a post-stocking movements study of 22 hatchery-reared juvenile pallid sturgeon (Jordan et al. 2006).

The riverine reach was divided into four sample zones of approximately equal length (Figure 1). The upper site extended from Fort Randall Dam (rkm 1,416) to downstream of Greenwood, South Dakota (rkm 1,392). The upper-middle site extended from Greenwood to near Verdel, Nebraska (rkm 1,377), the lower-middle site extended from Verdel to Running Water, South Dakota (rkm 1,360). The most downriver site encompassed the remainder of the river from Running Water to near Springfield, South Dakota (rkm 1,340). Extensive surveys of the Missouri River in all four zones occurred on four dates in 2000, 13 dates in 2001, and 10 dates in 2002. One survey date in 2002 occurred during the winter (January). The reservoir, Lewis and Clark Lake, was also searched once in 2000 and once in 2001. One final survey of the riverine reach was done in 2003 near the end of the battery life for the transmitters.

Two tracking methods, extensive and intensive, were employed during each sample period in 2000. Extensive tracking was also conducted throughout 2001 and 2002. Extensive tracking ascertained the locations of as many fish as possible in each zone by stopping and listening for fish about every 0.4 km. Then a randomly selected individual fish was intensively followed for 24 h. Intensive tracking encompassed the day, night, and both crepuscular periods, dawn and dusk. These diel tracking periods were defined as: dawn (1 h before to 1 h after sunrise), day (2 h after sunrise to 2 h before sunset), dusk (1 h before to 1 h after sunset), and night (2 h after sunset to 2 h before sunrise). Tracking began immediately after release and occurred approximately every other week, until weather conditions prohibited tracking during late fall and resumed as early as feasible each spring. One tracking survey occurred in winter (January 2002).

An ultrasonic receiver and directional hydrophone were used to detect fish locations and a fish was considered located when the coded impulses from the sonic transmitter were equally audible with a 360° rotation of the hydrophone. Once a fish location was determined, latitude and longitude coordinates were recorded with a PLGR+96 Global Positioning System (GPS) receiver (Rockwell
International, Milwaukee, Wisconsin). The mean error reading (N = 67) for GPS locations of adult pallid sturgeon from both extensive and intensive surveys was 5.2 m (SE = 0.2). The habitat type for each located fish was classified as: main channel, side channel, backwater, island tip, reservoir, tributary mouths, and dam tailrace. Habitat characteristics measured at each fish location included surface water temperature (ºC), water depth (m) occupied by the fish, maximum water depth (m) of the channel cross-section, bottom water velocity (m/s), and turbidity (nephalometric turbidity units, [NTU]). The relative depth for each fish was calculated as the ratio of the water depth at the fish’s location to the maximum water depth of the channel cross-section.

RESULTS

Post-surgery survival

During the five-week holding period, two mortalities (mean FL = 1,320 mm [SE = 50] and mean weight = 15.7 kg [SE = 2.8]) from the Lake Sharpe adult pallid sturgeon occurred within 24 h of tag implantation. Lengths and weights of dead fish were similar to those that survived for five weeks after surgery. No expulsions of tags were observed, but inflammation at the incision was noted. Four of the pallid sturgeon (mean FL = 1,322 mm [SE = 84] and mean weight = 14.8 kg [SE = 2.6]) stocked at Verdel, NE were relocated at least once during the first year, but only two fish (Fish 375 and Fish 446) were found in both 2001 and 2002, suggesting a minimum survival rate of 33% of the stocked fish (Table 1). Fish 446 was a male and the sex of Fish 375 was unknown. The two pallid sturgeon stocked at Running Water, SD were never relocated during the study.

Habitat

In all seasons, adult pallid sturgeon were located in the main channel habitat (mean depth = 4.1 m [SE = 0.3]), except on one occasion where a fish was found in a deep (4.1 m) secondary channel in the delta formed at the headwaters of Lewis and Clark Lake. However, the Missouri River below the Niobrara River Confluence (rkm 1,358) is dynamic, with continuously shifting channels and differentiation between secondary channels and the main channel was often difficult. Fish 375 was located in this dynamic area below the confluence during the entire study, whereas, over 53% of the relocations for Fish 446 were found above the confluence where there is a distinct main river channel habitat. No fish were found in the tailrace, island tips, backwaters, reservoir, or in tributaries. Relative depths where the two pallid sturgeon were relocated ranged from 79 to 100% of the maximum depth of the river. Turbidity in all years, seasons, and habitats was uniformly low (< 34 NTU). Bottom water velocities associated with pallid sturgeon locations ranged from 0.1 to 0.9 m/s. No substantial differences in depth, percent maximum depth, or turbidity were apparent among seasons (Table 2).
The two fish found in all years of the study demonstrated different ranges of movement. After stocking in 2000, Fish 446 moved upstream and Fish 375 moved downstream of the stocking site (Figure 2). The mean range of movement between successive relocations of Fish 446 during the extensive tracking was 11.5 km in 2000 (n = 2 observations), 27.1 km (SE = 9.1) in 2001 (n = 7), and 13.2 km (SE = 5.0) in 2002 (n = 8). The mean range of movement between successive relocations of Fish 375 was 1.3 km (SE = 1.1) in 2000 (n = 3), 2.6 km (SE = 2.5) in 2002 (n = 4), and 7.1 km in 2002 (n = 2). Substantial differences

### Table 1. Size at tagging, number of relocations, and days at large for adult pallid sturgeon implanted with ultrasonic transmitters and released in the Missouri River downstream of Fort Randall Dam on 6 July and 20 September 2000.

<table>
<thead>
<tr>
<th>Transmitter number</th>
<th>PIT tag number</th>
<th>Fork length (cm)</th>
<th>Weight (kg)</th>
<th>Date last seen</th>
<th>Days at large</th>
<th>Total relocations</th>
<th>Relocations by year</th>
</tr>
</thead>
<tbody>
<tr>
<td>244</td>
<td>7F7D291C3D</td>
<td>141</td>
<td>17.1</td>
<td>12 July 2000</td>
<td>36</td>
<td>1</td>
<td>1 0 0 0</td>
</tr>
<tr>
<td>375</td>
<td>7F7D396837</td>
<td>107</td>
<td>7.2</td>
<td>1 April 2003</td>
<td>1029</td>
<td>10</td>
<td>3 4 2 1</td>
</tr>
<tr>
<td>248</td>
<td>7F7D441774</td>
<td>144</td>
<td>21.1</td>
<td>20 Sept 2000</td>
<td>0</td>
<td>0</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>258</td>
<td>113719262A</td>
<td>137</td>
<td>9.8</td>
<td>20 Sept 2000</td>
<td>0</td>
<td>0</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>446</td>
<td>7F7D352F24</td>
<td>142</td>
<td>17.8</td>
<td>23 Sept 2002</td>
<td>839</td>
<td>17</td>
<td>2 7 8 0</td>
</tr>
<tr>
<td>2246</td>
<td>7F7D267960</td>
<td>139</td>
<td>17.4</td>
<td>13 July 2000</td>
<td>37</td>
<td>1</td>
<td>1 0 0 0</td>
</tr>
</tbody>
</table>

* Two fish were never relocated after release.

* Relocations in 2003 resulted from ad hoc sampling, near end of battery life for the transmitters.

### Table 2. Mean seasonal habitat characteristics with standard error (SE) in parenthesis where two adult pallid sturgeons implanted with ultrasonic transmitters and stocked in the Missouri River downstream of Fort Randall Dam, South Dakota and Nebraska were relocated from 2000 – 2003. N indicates combined relocations for both fish.

<table>
<thead>
<tr>
<th>Season</th>
<th>N</th>
<th>Temperature (°C)</th>
<th>Depth (m)</th>
<th>% max depth</th>
<th>Turbidity (NTU)</th>
<th>Bottom Velocity (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>6</td>
<td>6.7 (1.0)</td>
<td>4.1 (1.0)</td>
<td>92.0 (2.5)</td>
<td>14.7 (9.7)</td>
<td>No data</td>
</tr>
<tr>
<td>Summer</td>
<td>12</td>
<td>22.4 (0.7)</td>
<td>4.0 (0.4)</td>
<td>82.5 (4.7)</td>
<td>14.1 (2.7)</td>
<td>0.6 (0.2)</td>
</tr>
<tr>
<td>Fall</td>
<td>5</td>
<td>17.3 (1.6)</td>
<td>4.1 (0.3)</td>
<td>87.8 (6.2)</td>
<td>16.7 (10.0)</td>
<td>No data</td>
</tr>
<tr>
<td>Winter</td>
<td>2</td>
<td>1.3 (0.1)</td>
<td>3.7 (0.4)</td>
<td>94.6 (3.0)</td>
<td>No data</td>
<td>No data</td>
</tr>
</tbody>
</table>

Fish Movement

The two fish found in all years of the study demonstrated different ranges of movement. After stocking in 2000, Fish 446 moved upstream and Fish 375 moved downstream of the stocking site (Figure 2). The mean range of movement between successive relocations of Fish 446 during the extensive tracking was 11.5 km in 2000 (n = 2 observations), 27.1 km (SE = 9.1) in 2001 (n = 7), and 13.2 km (SE = 5.0) in 2002 (n = 8). The mean range of movement between successive relocations of Fish 375 was 1.3 km (SE = 1.1) in 2000 (n = 3), 2.6 km (SE = 2.5) in 2002 (n = 4), and 7.1 km in 2002 (n = 2). Substantial differences
in maximum observed range and seasonal movement rates were found between Fish 446 and Fish 375 (Table 3). Seasonal movement rates should be interpreted cautiously as considerable fish movement likely occurred during the time period between relocations of the fish.

Both fish showed similar seasonal movement patterns of moving upstream either during late fall or in the spring and then downstream during summer; however, the magnitude of these movements differed. Fish 446 was located throughout the study area and showed a pronounced seasonal pattern. In contrast, Fish 375 remained downstream of the stocking site in the delta formed below the Niobrara River Confluence and had a reduced seasonal pattern. Fish 446 moved upstream to main river channel crossover near rkm 1,407 on 13 July 2000 and returned to the same location on 10 May 2001. Within one month during the summer of 2001, this fish then moved 64 km downstream to the Bazille Creek Confluence. The following year, Fish 446 again moved upstream to a main river channel crossover near rkm 1,387, then moved downstream to the Bazille Creek Confluence where it was located the previous summer and subsequently returned to the same upstream location observed in 2001. Fish 375 was commonly relocated at two distinct sites: 1) just upstream of Running Water (rmk 1,353) (16 August 2000 to 9 May 2001), which is characterized by
a dynamic area of shifting sandbars attributable to the influence of the Niobrara River’s sediment load and 2) just downstream of the Bazille Creek Confluence (9 July to 8 August 2001).

Diurnal movement rates were measured on four occasions. Fish 244 was intensively tracked only on 3 August 2000 with a mean movement rate of 0.83 ± 0.16 km/h. Fish 244 was never relocated throughout the rest of the study and likely either died or left the study area. Fish 375 was intensively tracked once in August, September, and October in 2000. The data for diel mean movement rates for Fish 375 were pooled. Movement rates were substantially higher (≥ 40%) at night compared to dawn, daytime, and dusk diel periods (Table 3). The maximum movement rate was 0.15 km/h during the day and the minimum was also during the day at 0.002 km/h.

Clusters, defined as ≥ 2 fish within 750 m of each other (Jordan et al. 2006), of an adult and a juvenile pallid sturgeon were observed once on 23 July 2002. Evaluation of extensive tracking data (29 relocations) indicated Fish 375 and 446 were never observed together throughout the study.

**DISCUSSION**

Our transmitter weight to fish body weight ratio never exceeded the 2% recommended by Nielsen (1992). However, the two observed mortalities were likely due to the combined stress of surgery and elevated water temperatures. Higher water temperatures have been reported to increase stress and mortality for many fish species (Walsh et al. 2000; Davis 2004; Meka and McCormick 2005). Increased infection was also reported after implantation of a transmitter at elevated water temperatures (Walsh et al. 2000). The six pallid sturgeon healed from the transmitter implantation surgery but had some inflammation around the incision at time of stocking. Warm water temperatures during surgery may have increased stress and infection, but warmer water temperatures also promotes rapid healing in sturgeon (Herb Bollig, U.S. Fish and Wildlife Service, Gavins Point NFH, personal communication; 2005). Ream et al. (2003) also

### Table 3. Range of movement (in river km) and seasonal movement rates (km/d) with standard error (SE) in parenthesis of two sonic-tagged adult pallid sturgeon from 2000 to 2003. Diel movement rates were from Fish 375 that was intensively tracked once in August, September, and October in 2000 in the Missouri River downstream of Fort Randall Dam, South Dakota and Nebraska.

<table>
<thead>
<tr>
<th>Fish number</th>
<th>Maximum range (rkm)</th>
<th>Seasonal movement rate (km/d)</th>
<th>Diel movement rate (km/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spring</td>
<td>Summer</td>
</tr>
<tr>
<td>375</td>
<td>8.1</td>
<td>No data</td>
<td>0.04 (0.03)</td>
</tr>
<tr>
<td>446</td>
<td>45.8</td>
<td>1.11 (0.47)</td>
<td>0.71 (0.19)</td>
</tr>
<tr>
<td>244</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
</tr>
</tbody>
</table>

*a Defined as the difference from the most upstream and downstream locations.

*b Single observation of movement rate for Fish 375 in Fall 2000.
reported increased wound healing in warmer water temperatures among four teleost fish adapted to widely different thermal environments. Tews et al. (1994) found the incision wound on one adult pallid sturgeon, captured and tagged in the cold waters of the tailrace of Fort Peck Dam in Montana during July, failed to heal completely after five months. Snook et al. (2002) reported 50% of their fish shed tags or presumably died after release in the Platte River, Nebraska in April using radio tags that weighed < 1.2% of the body weight (1.2 – 2.5 kg) of age-6 juvenile pallid sturgeon. Jordan et al. (2006) also reported 56% mortality of age-3 juvenile pallid sturgeon when sonic transmitter weight to fish body weight ratios ranged from 2.1 to 3.5%. Anesthesia during surgery may reduce stress levels and increase post surgery survival. Hurley (1999) anesthetized adult pallid sturgeon with CO2 (85 L/min for 3 - 6 min) before surgery and subsequently relocated 75% of the fish 100 d after release. Fish in this study, Jordan et al. (2006), and that of Snook et al. (2002) were not anaesthetized while the tags were inserted. Based on recent experiments, the USFWS (2006) recommends a safe and effective anesthetic during surgery on pallid sturgeon may be MS-222 in water buffered with sodium bicarbonate.

Habitat

Throughout this study, adult pallid sturgeon were almost exclusively located in the main channel with only one relocation in a deep secondary channel. However, one of the adult pallid sturgeon was located below the Niobrara River Confluence where differentiation between the main river channel and secondary river channels were often difficult due to the dynamic nature of shifting sandbars and numerous river channels. The high sediment load of the Niobrara River is deposited downstream of the confluence in the headwaters of the Lewis and Clark Lake creating a dynamic braided delta. Juvenile pallid sturgeon were also reported to use almost exclusively the main river channel in this reach of the Missouri River (Jordan et al. 2006). In the Middle Mississippi River, Hurley et al. (2004) found that adult pallid sturgeon used the main channel (39% of all relocations) despite negative selection for this habitat, but fish positively selected main channel border habitats (26%). In the Platte River, Snook et al. (2002) relocated juvenile pallid sturgeon adjacent to the main channel characterized by sharp changes in depth, but never found pallid sturgeon directly in the main channel. Bramblett and White (2001) also found that adult pallid sturgeon in the Yellowstone and Missouri rivers in Montana and North Dakota selected sinuous and dynamic river reaches with many islands and secondary channels. Middle Mississippi River adult pallid sturgeon selected for more diverse habitats downstream of island tips and wing dams (Hurley et al. 2004).

Pallid sturgeon are generally associated with deep turbid waters in the main channel of large rivers (Kallemeyn 1983). All adults in this study were predominately found in the main channel at relative depths > 79%. Juvenile pallid sturgeon in this same reach also were reported at relative depths > 80% (Jordan et al. 2006). Adult pallid sturgeon occupied somewhat smaller relative depths in the Upper Missouri River which may be due to low water clarity (Bramblett and White 2001). However, both juvenile and adult pallid sturgeon have been
located in shallow waters < 1 m (Bramblett and White 2001; Snook et al. 2002). Water clarity in the Missouri River below Fort Randall Dam was uniformly high (< 34 NTU) throughout our study which may account for the greater relative depths occupied by adult pallid sturgeon compared to adults in the Missouri and Yellowstone rivers (Bramblett and White 2001). Foraging for prey fish at greater depths and less light may be advantageous for pallid sturgeon in a river with low turbidity. Adult pallid sturgeon in Lake Sharpe, South Dakota were most commonly located in waters with bottom turbidities > 80 NTU (Erickson 1992).

In October 2002, the U. S. Geological Survey collected bathymetric data and used side scan sonar to collect images of habitat features where Fish 446 and 12 sonic-tagged juvenile pallid sturgeon were relocated as well as in unoccupied areas to assess habitat availability and selection (Elliot et al. 2004). They found that Fish 446 and the juvenile pallid sturgeon selected large sand dunes in the main channel over any other habitat available in the Missouri River downstream of Fort Randall Dam (Elliot et al. 2004; Jordan et al. 2006).

Fish Movement

Interestingly, both adult pallid sturgeon moved up and downstream returning to what may be two or three preferred locations throughout the study reach. These fish were likely finding favorable foraging areas during the different seasons. Returning to known areas of favorable foraging would be bioenergetically profitable, reducing the time needed to search for prey. However, these movements may have been related to spawning migrations. Movements upstream were either in the late fall or early spring while downstream movements occurred in summer. This study and past telemetry studies (Hurley 1999; Bramblett and White 2001; Snook et al. 2002) have described the high mobility of pallid sturgeon. However, one of our adult pallid sturgeon, Fish 446, had a substantially higher movement range and rates among all seasons compared to the other fish. Fish 446 was a known male and may have made a spawning migration during the study. The sex of Fish 375 was unknown and may not have made a spawning migration during the study. Gamete development in pallid sturgeon is extended over several years with an estimated 3 to 5 year interval between spawning for females, compared to only 2 to 3 year intervals for males (Herb Bollig, U.S. Fish and Wildlife Service, Gavins Point NFH, personal communication; 2006). The extent of annual movement by most adult pallid sturgeon in the Missouri and Yellowstone rivers in Montana was 20 – 80 km, but some fish moved distances > 200 km during spring and summer (Bramblett and White 2001). The mean extent of movements by adult pallid sturgeon in the Mississippi River was 34 km but this was considered a minimum estimate because searches for fish generally occurred within a 60 km reach of the river (Hurley 1999). It has also been reported that adult pallid sturgeon below the confluence of the Missouri and Yellowstone rivers had directed upstream movements into the Yellowstone River during spring (Bramblett and White 2001). However, middle Mississippi River adult pallid sturgeon moved both up and downstream during spring (Hurley 1999).
We did not find any substantial differences in daily movement rates among seasons for either fish but our sample size was small. However, Bramblett and White (2001) and Erickson (1992) reported adult fish in the Missouri River moved up to 21 km/d and were most active in spring. In Lake Sharpe, mean movement rates of adult pallid sturgeons were > 2 km/d from June through August compared to < 1 km/d during the remainder of the year (Erickson 1992). Hatchery-reared juvenile pallid sturgeon stocked below Fort Randall Dam in 2000 were more active in spring and fall compared to summer (Jordan et al. 2006). Movement rates of hatchery-reared juvenile pallid sturgeon in the Platte River ranged from 0.2 – 3.3 km/d (Swigle 2003). Movement rates in this study from extensively tracked fish surveys at two week intervals would not account for fish movement that occurred between surveys. Adult pallid sturgeon activity was likely underestimated if a fish returned to the same general area it was located previously, which may explain these lower movement rates compared to fish that were intensively followed for 24 h.

We found that Fish 375 moved more at night compared to dawn, daytime, and dusk. However, movement rates of hatchery-reared juvenile pallid sturgeon in this same reach of the Missouri River did not significantly differ among diel periods (Jordan et al. 2006). Bramblett and White (2001) reported a greater proportion of adult pallid sturgeon moved during the day (56%) compared to night (37%) and hypothesized that pallid sturgeon would become more nocturnal in low turbidity habitats. They reported an average secchi depth of 20 cm. This hypothesis may explain the higher movement rates of an adult pallid sturgeon during the night in the low turbidity downstream of Fort Randall Dam. Erickson (1992) also reported significantly higher mean movement rates of adult pallid sturgeon at night (0.49 km/h) compared to during the day (0.23 km/h) with a mean turbidity of 91 NTU and mean secchi disk depth of 46 cm at adult pallid sturgeon relocations. Additionally, Hurley (1999) did not report water clarity, but reported that mean 2-hr movement rates for adult pallid sturgeon in the Middle Mississippi River were significantly greater during the day compared to night where turbidity is substantially higher compared to downstream of Fort Randall Dam.

We did not relocate the two adult pallid sturgeon within 1 km of each other throughout the study. Because of the low sample size and large study area (76 km), we are not suggesting that adult pallid sturgeon do not cluster or aggregate. Jordan et al. (2006) reported that on 15 dates, juvenile pallid sturgeon would cluster (≥ 2 fish within 750 m of each other) and these clusters were found in all seasons. Adult sturgeon also aggregated in spring and summer in the Missouri and Yellowstone rivers (Bramblett and White 2001). Although, Erickson (1992) did not consider pallid sturgeon to aggregate in a Missouri River reservoir, > 2 adult fish were found within 0.8 km of each other on four dates. These observations by Erickson (1992) are notable in context of the large study area (137 km length) and the small number of fish (n = 7) used. Spring and summer aggregations by adult pallid sturgeon may indicate fish congregating in spawning habitat, areas of high prey availability, or areas of refuge from high water velocities.

We recognize that our study was limited by small sample size (n = 2 fish). However, in the case of the pallid sturgeon, an endangered species, all field ob-
servations merit documentation to provide managers as large of a knowledgebase as possible to enhance recovery efforts. Overall this study found adult pallid sturgeon, like hatchery-reared juveniles (Jordan et al. 2006) moved throughout the remnant riverine Missouri River downstream of Fort Randall Dam known as RPMA 3 in the recovery plan (USFWS 1993). Wanner (2006) indicated RPMA 3 is suitable habitat for juvenile pallid sturgeon but whether this area will support adults and provide conditions needed for natural recruitment leading to self sustaining populations remains unknown.

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LITERATURE CITED


DISTRIBUTION OF TWO ARBOREAL SQUIRRELS IN ISOLATED FORESTS OF SOUTH DAKOTA

A. M. Kiesow
Department of Biology
University of South Dakota
Vermillion, SD, 57069

M. J. Hough
Department of Biology
South Dakota State University
Brookings, SD, 57007

J. A. Kiesow
Lower Brule Sioux Tribe
Department of Wildlife, Fish and Recreation
Lower Brule, SD 57548

ABSTRACT

Northern flying squirrels (*Glaucomys sabrinus*) and red squirrels (*Tamiasciurus hudsonicus*) are found in the Black Hills of South Dakota and Wyoming (Wells-Gosling and Heaney 1984, Jones et al. 1985), but their occurrence in other forested regions of South Dakota remains in question. We sampled and surveyed forested regions in northwestern and northeastern South Dakota to determine whether these two squirrel species were found in other forested regions of South Dakota, where they were historically reported. Sampling was undertaken by placing traps and conducting random site surveys and searching for sign in the respective regions. Northern flying squirrels were not detected in either the northeast or northwest region. Red squirrels were detected in the northeast but not the northwest region of South Dakota. These areas should be trapped and surveyed more extensively in the future, focusing efforts in the northeast region of South Dakota.

Keywords

Arboreal squirrels, northwestern South Dakota, northeastern South Dakota, northern flying squirrel, red squirrel

INTRODUCTION

Currently northern flying squirrels (*Glaucomys sabrinus*) and red squirrels (*Tamiasciurus hudsonicus*) are distributed in the Black Hills of South Dakota and Wyoming (Wells-Gosling and Heaney 1984, Jones et al. 1985), but there may also be small populations in other forested regions of South Dakota, particularly
in the northeast (Over and Churchill 1941, Wells-Gosling and Heaney 1984; Figure 1). Populations of these squirrels are likely small and somewhat rare and considered disjunct from other populations within their range (Jones et al. 1985).

There are data gaps regarding both squirrel species outside the Black Hills. Little is known about the population of northern flying squirrels in northeastern South Dakota. Over and Churchill (1941) detected flying squirrels near Big Stone Lake in northeastern South Dakota. They considered this population comprised of southern flying squirrels (G. volans). Wells-Gosling and Heaney (1984) reported that this population is comprised of northern flying squirrels. Red squirrels were once reported in northeastern South Dakota, although they do not appear to be restricted to this area historically (Turner 1974). Red squirrels, though probably found in coniferous areas of western South Dakota (Visher 1914), are limited to the dense ponderosa pine forests of the Black Hills (Jones et al. 1985). Nonetheless, red squirrels were discovered several miles to the west in eastern Montana in relatively dense stands of ponderosa pine of Long Pine Hills (Anderson and Jones 1971).

Both squirrel species are known to reside in the Black Hills, but it remains uncertain as to whether each species is present in other forested regions of South Dakota, in particular northwestern and northeastern South Dakota. These areas include coniferous forests of Custer National Forest, Sioux Ranger District in northwestern South Dakota and old riparian and coulee deciduous forests of northeastern South Dakota. Our objective was to determine the presence/absence of these two species in these two respective regions of South Dakota.

Figure 1. Historical distribution of northern flying squirrels (left) and red squirrels (right) in South Dakota.

STUDY AREA AND METHODS

The study areas were the Slim Buttes of Custer National Forest in northwestern South Dakota, the coulees of Sica Hollow State Park, and the riparian forests of Lake Traverse and Big Stone Lake in northeastern South Dakota (Figure 2).

Trapping occurred from 27 May to 2 June 2006 (or six trap nights) in the Slim Buttes in northwestern South Dakota. This area was selected because it
represented the largest coniferous forest in this region. We did not survey the Cave Hills of Custer National Forest because there was little forested habitat. The Short Pine Hills was not sampled because accessibility was difficult.

Trapping occurred from 13 to 24 December 2004, 27 to 31 December 2004, and 24 to 29 July 2006 (or 20 trap nights) in coulees of Sica Hollow State Park and from 24 to 29 July (or five trap nights) in riparian forests in northeastern South Dakota (i.e., Lake Traverse and Big Stone Lake; Figure 2). These areas were selected because they represented two different forested regions of northeastern South Dakota: 1) coulee forests and 2) riparian forests.

We established four line transects in suitable habitat (i.e., forested areas along ridges) in both study areas. Ten Tomahawk® and/or Havahart® traps (specific to trapping squirrels) were placed in each line transect and spaced 60 to 80 meters apart. The traps were baited with a mixture of bacon grease, oatmeal, and peanut butter and were checked and re-baited (if needed) each day.

If a squirrel was captured, we recorded the species, sex, weight, date, time of day, temperature, cloud cover, and wind speed. We also collected a tissue sample. Captured squirrels were marked under the chin with a paint pen and released.

In addition to trapping, general observations (i.e., presence of snags, presence of red squirrel food caches [or middens], presence of dray nests, visual observations of each species, and presence of flying squirrel food resources) were made along each respective transect.
RESULTS

No squirrels were captured in northwestern South Dakota, and neither species was observed during the trapping period. In addition, no dray nests were recorded and no food caches were detected for red squirrels along each transect. However, there were greater than ten snags per transect, and we observed numerous fungi for flying squirrels as well as fruits/nuts for red squirrels.

No squirrels were captured in northeastern South Dakota during the summer and winter trapping periods, but red squirrels were observed/heard in the riparian areas of Lake Traverse and Hartford Beach State Park (at Big Stone Lake) during the summer trapping period (Figure 3). In addition, red squirrel middens were noticed near transects located in the riparian areas. There were also greater than ten snags available for roost/nest sites along each transect, but no dray nests were noticed. Our results show that red squirrels still are present in northeastern South Dakota.

DISCUSSION

Forested regions in South Dakota were fragmented as a result of past geological events, such as glaciations, and other factors, such as habitat degradation (Turner 1974). Coniferous forests in northwestern South Dakota (largely ponderosa pine) are discontinuous with other coniferous forests. Deciduous forests in northeastern South Dakota are also fragmented but contain a mixture of maple, ash, elm, basswood, and walnut trees in riparian areas and coulees. The forests in northwestern and northeastern South Dakota are isolated and may support species able to disperse long distances, e.g., birds (Medellin and Gaona 1999).

Arboreal mammals (i.e., red squirrels and northern flying squirrels) likely have difficulty with long distance dispersal because of their dependence on trees for travel, food, and rest (Sun 1997, Vernes 2001). As a result, isolated forests in South Dakota likely have lower mammalian diversity compared to continuous
forested regions in South Dakota (e.g., Black Hills). In addition, isolated forests may not be large enough to sustain viable species populations. Studies have shown certain species may require a certain size habitat to sustain the population (Michalski and Peres 2005). Northern flying squirrels are no longer found in northeastern South Dakota and may have never been present in northwestern South Dakota, whereas red squirrels are no longer found in northwestern South Dakota but still reside in northeastern South Dakota. This is likely the result of their poor ability to disperse and the patchiness of the forest habitat.

Moreover, vicariance could have played a role in the isolation of forests and arboreal mammals, where once widespread arboreal mammals were isolated to forests that remained after the glaciers receded during the Pleistocene (Lomolino et al. 2006). We suspect that if these squirrel species were isolated to these forests that they eventually were extirpated in this region due to genetic drift, climate changes, or other factors (e.g., loss of habitat) being that neither species was detected in the northwest and only red squirrels were detected in the northeast. Red squirrels are still found in the northeast, but this population is likely small and slowly decreasing in size as a result of further fragmentation of forested habitat and thus isolation in this region.

There are other isolated forests in South Dakota, such as ponderosa pine forests in Pine Ridge Indian Reservation, Rosebud Indian Reservation, and White Owl, which may support northern flying squirrel or red squirrel populations. These isolated forests should be targeted for further surveys. In addition, we recommend additional trapping periods over several years to determine more about the status and distribution of arboreal mammals in northwestern and northeastern South Dakota.

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LITERATURE CITED


COMPARISONS OF THE NATIONAL WEATHER SERVICE COOPERATIVE OBSERVER PROGRAM (COOP) AND THE SOUTH DAKOTA AUTOMATED WEATHER DATA NETWORK (AWDN) MAXIMUM AND MINIMUM TEMPERATURES IN SOUTH DAKOTA

J. Puetz Anderson, D.P. Todey and Chirag Shukla
Agricultural and Biosystems Engineering
South Dakota State University
Brookings, SD 57007

ABSTRACT

In South Dakota, National Weather Service Cooperative Observer Program (COOP) volunteers have been collecting daily weather data for more than a century. All 150 sites report precipitation amount and type; a subset of these sites also record maximum and minimum temperatures. A few sites, add 24 hour wind run, and evaporation. In the mid-1980s, South Dakota began deploying Automated Weather Data Network (AWDN) to complement the COOP network. Today thirty sites automatically report hourly weather data. The use of two systems has necessitated questioning of how the data from the AWDN compare to the nearby COOP station data.

The AWDN system reports the average, maximum and minimum temperature and relative humidity for each hour plus hourly radiation, average wind speed, direction, gusts and rainfall amount. This data is typically reported midnight to midnight in daily summaries. Excel spreadsheets were used to restructure the hourly AWDN data to match the same 24 hour day as the nearest COOP station. Then statistical comparisons were performed on the temperature data.

Sites closer in geographic proximity produced stronger correlations in the data. The daily temperature differences between the South Dakota AWDN and the COOP for each set of paired data had standard deviations that ranged from 0.49 to 1.89°F per day with an $R^2$ (coefficient of determination) greater than 0.99 at Brookings, where the sensors are less than 3 meters apart. Chamberlain and Oacoma sites are located 19 kilometers apart. The standard deviations for this pair were 1.91 °F for daily maximum temperatures (Tmax) and 2.88 °F for daily minimum temperatures (Tmin), $R^2$ was 0.98.

Using these comparisons, estimates can be made to replace missing data. The networks can be integrated, producing a finer resolution system improving the spatial depiction of weather and climate across South Dakota. These comparisons can be done automatically and could lead to an almost continuous instrument performance evaluation between scheduled calibrations.
INTRODUCTION

Cooperative Observer Program

The National Weather Service Cooperative Observer Program (COOP) has been the main climate data source for over a century in South Dakota. The daily reports are for the periods of 24 hours, but are taken at a non-uniform time, some AM, some PM, and some at midnight. Many COOP volunteers are of retirement age living in small towns with few people. Sometimes the data are slow to be reported.

All sites report precipitation amount and type. Others also include maximum and minimum temperatures in Fahrenheit. A few sites include evaporation and wind run measurements. Presently, South Dakota has over 150 COOP sites. The data from 30 of these sites is on the Internet within a few hours of when the observations were taken. The data from the remaining sites are sent in as data sheets to NWS offices via United States mail. The COOP weather data are reported on the Internet at South Dakota Climate and Weather Ag Data whose website address is: http://climate.sdstate.edu/climate_site/ag_data.htm.

Automated Weather Data Network

In the mid-1980s, need for more detailed automated data led to the deployment of the South Dakota Automated Weather Data Network (AWDN). Currently, about thirty sites in South Dakota automatically report hourly or sub-hourly weather data with one set up this summer, and a couple more planned for the future. The South Dakota AWDN stations record data every 5 seconds, reporting summaries every 5 minutes, each hour, and each day. Data reported are the average temperature (°F) for the hour and the maximum and minimum temperature (°F) sampled every 5 seconds for the hour. The average, maximum and minimum hourly relative humidity (%), hourly radiation (w / m²), average wind speed (mph), direction (degrees azimuth) and gusts, and rainfall (inches) are also reported. Currently, the AWDN rain gauges can only measure liquid, not frozen precipitation.

COOP and AWDN Comparisons

The AWDN hourly observations are converted into a daily summary, where the 24 hour day goes from midnight to midnight. The daily AWDN summaries are available from the Internet. The COOP reports daily observations, 8 AM to 8 AM. Because the two systems often use a different 24 hours for their daily observations, comparing their daily data as is would not produce useful information.
For this comparison, the first step was to put the daily weather observations on the same 24 hour period to allow proper statistical analysis to be performed. The results of these statistical comparisons will illustrate how gathered data can be used between the two networks.

In the future, NOAA plans to upgrade the COOP network. All automated COOP sites will provide baseline measurements of temperature and precipitation. Data transmission will occur in real-time at hourly intervals with a goal of transmitting 5-minute observations at 15-minute intervals. Many of the newly automated COOP sites will have human observers who will have the ability for real-time transmission of manually entered data. (NOAA, 2004) When the COOP sites are upgraded to automated systems, this study's restructuring methods and correlation information could be used to create the historical COOP data format from updated automated COOP stations that will report hourly and sub-hourly summaries. Secondly, this analysis could also be used to see how well the two systems' data can be integrated into a common map. Thirdly, the calculated regression relationships between the sites could be used in two ways; 1) to calculate an estimate from one site to another on the occasions when there may be missing observations, 2) to develop a mathematical model that would be able to calculate the differences between two sites. This diagnostic tool would monitor the performance of the observations sites continuously and automatically. The model would flag data if differences larger than a predetermined amount were observed. For Brookings, if differences 1 ºF or more are observed; this data would be flagged for further investigation. Because the COOP data are reported in whole degrees Fahrenheit, 1 ºF or greater was chosen to be flagged as the minimum resolution difference for Brookings where the sensors are so close. Other paired sites that are farther apart may have a larger value before the data would be flagged.

METHOD

Because the NWS official data are collected and reported in degrees F, Fahrenheit was used throughout the paper. Distances are in km and meters with English amounts in parentheses. For this study, the 8 AM observed Tmax and Tmin at the COOP site at Brookings were compared to the 24 hour 8am to 8am calculated AWDN Tmax and Tmin for the years of 2003 - 2006. Temperature data were also compared at three paired locations for the year 2004; Brookings COOP and AWDN, Chamberlain COOP and Oacoma AWDN, and Centerville 6SE COOP and Beresford AWDN.

The three COOP locations in this study all used an 8 AM to 8 AM observation time. In order to obtain useful information on how well the data from the two networks compared, the AWDN data needed to be converted to the same 24 hour time period as the nearest COOP site used. Excel spreadsheets were used to restructure the hourly AWDN data into the same 24 hour day as the nearest COOP station. Datasets were extracted from the South Dakota Climate and Weather website.
The hourly AWDN data from 9 AM Dec 31 one year to 8 AM Dec 31 the next year were placed on a work sheet. Missing or extra data were flagged. Duplicate observations were dropped and missing data were replaced with interpolated estimates that would not change either the Tmax or Tmin which existed in the observed hourly data for that date. The next step was to find the daily Tmax and Tmin during the 24 hour time period using the 9 AM to 8 AM hourly observations for each day of the year. The third step was to make sure there were no missing COOP daily reports. If there was a missing COOP report, only the date was entered so that the daily AWDN values would line up with the daily COOP observations for the same day. The differences between the two stations were calculated for each day of the year. Because the COOP is the accepted historical climate observation network the COOP temperatures were subtracted from the AWDN temperatures.

Equation 1. \( \Delta T = \text{AWDN temperature} - \text{COOP temperature} \)

Using Excel, graphs were plotted and the coefficient of determination and standard deviations were found for these calculated differences. The next step was to calculate a regression equation for all paired sites and years. These equations were used to estimate a realistic value for a missing observation from one site’s data using the observation from the other station for that day, and finally to determine if a diagnostic model could be created from this information to evaluate the data from these two systems to check the performance of the instruments on an almost real-time basis. Figure 1 is the graph and regression equation

**Brookings Minimum Temperatures for 2004 - 2006**

\[ y = 0.9956x + 0.1454 \]

\[ R^2 = 0.9969 \]

*Figure 1. Scatter plot of compared minimum temperatures for Brookings (2004 - 2006).*
for Brookings 2004 through 2006. The data from Brookings for the years 2004-
2006 were combined into one data set to calculate a regression equation. The
reason these three years were used to create the model was they had similar \( R^2 \) values.

The 2003 COOP data and the three year regression equation were used to
create a mathematical model to estimate AWDN temperatures for 2003. The
actual AWDN data was subtracted from the estimated values and a new scatter
plot and standard deviations and \( R^2 \) were calculated for the differences between
the estimated and the actual temperatures for the year of 2003.

RESULTS

Of the weather data collected, temperature is usually the most spatially con-
sistent and conservative. Therefore, there should be a strong correlation between
the two systems' daily T max and T min, since distances between sensors were 3
meters (10 feet) up to 19 km (12 miles) which is less than 30 km for T min's and
60 km for T max's. (Hubbard, 1993) In the mid 1990’s Hubbard did research to
find how far apart in the plains each of the reported weather variables could be
and have an \( R^2 \) of 0.90 or greater. To explain more than 90% of the variation
in maximum temperatures between sites, a spacing of 60 km is sufficient on a
year-round basis. Minimum temperature, relative humidity, solar radiation, and
evapotranspiration require closer spacing (≤ 30 km) to achieve this criterion…
(Hubbard, 1993) The \( R^2 \) values would also be expected to improve as shorter
distances separate sites without significant topographic differences. For each set
of data that was compared the daily T max values had a higher \( R^2 \) than the daily
T min values. The difference between the COOP and AWDN daily T max for
Brookings was less than 1 degree Fahrenheit most of the time (Fig. 2). In 2004
there were only 6 times the differences between the COOP and AWDN daily
T max were greater than 1 °F. Figure 3 shows the daily T min differences. There
were 57 times when the T min differences were greater than 1 °F (Fig. 3). The
values of the differences show a greater variability about the mean in Figure 3 for
T min than what appears in Figure 2 for T max.

The South Dakota restructured AWDN 24 hour observation period ends
exactly 8 AM. The COOP 24 hour period begins and ends at 8 AM ± 10-15
minutes. It is rare for the high temperature of the day to occur near this time. So
the 10-15 minute variation in recording 24 hour observation, does not affect the
daily T max observed but may affect the T min. Also T min occurs closer to 8 AM
sometimes of the year more often than others. This would lead to seasonal and
Daylight Saving Time variations in T min differences between the COOP and the
AWDN observations, which could explain, part of the greater variability with the
daily T min than the daily T max values between the COOP and AWDN sites.
The relative abundance of temperature networks and the lack of information on
spatial variability may be the result of non-homogeneity in the data resulting
from difference in time of observation (Karl et al., 1986). An automated station
is programmed to take measurements on a fixed schedule and the same technique
is employed for all sites in the network. This eliminates the site-to site variability
often associated with a human observer. (Hubbard, 1994)
Figure 2. The difference in the daily maximum temperatures between the Brookings COOP and AWDN in 2004.

Figure 3. The difference in the daily minimum temperatures between the Brookings COOP and AWDN in 2004 (note there is more variability than on Figure 2).
As expected the closer the paired sites were geographically the stronger the statistical correlations. At the Brookings sites where the two thermometers were less than 3 meters (10 feet) apart, the standard deviations for the differences between the COOP and AWDN temperature differences ranged from 0.49 to 1.89 °F per day. The \( R^2 \) was better than 0.99. Figure 5 is an example of a scatter plot from the Brookings site. The annual means each year from the Brookings data sets showed a slight bias. The AWDN’s minimum temperatures were slightly higher than the COOP’s minimum temperatures and the AWDN’s maximum temperatures were slightly lower. One reason for this could be attributed to the height of the AWDN temperature sensor being slightly higher above the ground than the COOP sensor, because the ground is a heat source and sink for air. The COOP sensor being closer to the ground would allow for slightly warmer highs and slightly cooler lows to be observed. The annual means of the temperature difference for the two networks ranged from -0.14 to 0.13 °F per day for lows, and -0.07 to -0.04 °F per day for the highs. Relative location to the ground could also contribute to a larger temperature difference between the two sensors for morning lows versus afternoon highs, since there is often less wind in the morning to mix the air. Another reason could be, the way the temperatures are sampled. The COOP keeps track of the T max and T min instantaneous throughout the day. The AWDN takes a sample of the temperature every 5 seconds and reports that. This means the COOP would catch lower T min’s and higher T max’s than the AWDN. Additional years of data would be required to prove this.

Chamberlain and Oacoma sites were located 19 kilometers (12 miles) apart. The standard deviations of the differences between this pair of sites were 1.91 °F per day for maximums and 2.88 °F per day for minimums. \( R^2 \) was 0.97 or
higher for 2004. Figure 6 shows the scatter plot for Chamberlain and Oacoma daily Tmax and the $R^2$ as 0.99. Figure 7 displays Chamberlain and Oacoma daily Tmin’s with $R^2$ values better than 0.97. On Figure 6 there was more variability about the line of regression than Figure 5 where the sensors are located less than 3 meters (10 feet) apart. This is due to the close proximity of the Brookings sensors compared to Chamberlain and Oacoma paired sites. As with Brookings the variation in the difference of Tmin of Chamberlain and Oacoma in Figure 7 is greater than the variation in the difference of Chamberlain and Oacoma Tmax in Figure 6.

The correlations between daily Tmax differences were higher than that for the daily Tmin’s for all paired observations sites. This was seen for all the paired sites in coefficients of determinations (Fig. 8). In order to obtain comparable standard deviations the days when there were missing COOP data in 2006, those dates were removed from the calculations in (Fig. 9) that column is referred to as 2006 w/o. Standard deviations were smaller for the Tmax’s than the Tmin’s.

The differences between the Tmax and Tmin were most pronounced at the Chamberlain and Oacoma sites, where the sites were 19 kilometers (12 miles) apart and there were topographic differences. This would accentuate the differences because of cold air drainage even in subtle low areas.

In Figure 2 and Figure 10 there appears to be a seasonal relationship for 2004. Figure 10 uses the same data as Figure 2 but is a column graph of the monthly average of the daily differences in the Brookings 2004 COOP and
Figure 6. A scatter graph and regression equation of 2004 Chamberlain COOP compared to Oacoma AWDN daily maximum temperatures (Note there is less variability around the regression line Figure 4 for Brookings Tmax than on Tmax Figure 5 for Chamberlain and Oacoma)

Daily Maximum Temperatures
Chamberlain COOP and Oacoma AWDN (2004)

\[ y = 0.9722x + 1.5908 \]
\[ R^2 = 0.993 \]

Figure 7. A scatter graph and regression equation of 2004 Chamberlain COOP compared to Oacoma AWDN daily minimum temperatures.

Daily Minimum Temperatures
Chamberlain COOP compared to Oacoma AWDN (2004)

\[ y = 1.007x + 0.2481 \]
\[ R^2 = 0.9791 \]
Figure 8. Coefficients of Determination for the years and site studied. The first four sets of columns are the 4 years studied for Brookings, C&B is Centerville and Beresford for 2004, C&O is Chamberlain and Oacoma for 2004, and B model 2003 is the $R^2$ for the AWDN estimates created from the 2003 COOP data compared to the AWDN actual temperature values for 2003.

Figure 9. The first four sets of columns are Brookings standard deviations in degrees Fahrenheit of the differences between the COOP and the AWDN data sets for the daily highs and lows. The fifth is 2006 Brookings data without the March 11, 2006 data removed because the COOP observation was missing for that date. The 6th column, C & B is the standard deviations for Centerville and Beresford for 2004. And the last column, C & O is Chamberlain and Oacoma 2004.

AWDM data sets. Figure 10 demonstrates a positive difference in the cold season and negative difference in the warm season. This seasonal pattern does not seem to appear in Figure 3. There needs to be further investigation into the why this occurs, and if there are other years this happens. Because this is a single year occurrence more work will need to be done to determine potential cause for this.

DISCUSSION

The Brookings COOP and the ADWN hourly datasets restructured to the COOP 8 AM 24-hour observation period were compared with the R² values of 0.99 or greater. These results indicate that AWDN hourly observations could be converted into daily Tmax and Tmin to match the official historical COOP 24 hour format. In the future when COOP stations have their temperature sensors upgraded to hourly automatic summaries, this hourly information can be restructured into the official historical COOP 24 hour format of each site. This could maintain the continuity of the data for long-term climate research, while the hourly and sub-hourly data would better serve weather users real-time needs. Because of the slight difference between the Brookings COOP and AWDN Tmax and Tmin values apparently being related to sensor height differences, when the COOP sensors are upgraded to automatic systems, efforts should be made to insure the new instruments are placed at the same height above the surface. The sensors should also have the same sensitivity as the previous sensors.
to assure the integrity of the new COOP daily Tmax and Tmin data with the historical COOP data.

COOP and AWDN weather stations in Brookings were installed within 3 meters (10 feet). Maximum and minimum temperatures from these stations display strong correlations with $R^2$ values greater than 0.99. The annual means of the daily COOP and AWDN temperature differences were ±0.14 and ±0.7 (°F per day) for Tmax and Tmin respectively. Similar results were obtained when the Centerville 2SE’s COOP and Beresford AWDN paired sites and Chamberlain COOP and Oacoma AWDN paired sites were compared. Because such high correlations were found, the comparison information can be used in two ways. First, on the occasions when data is missing one or more sites could be used to calculate a realistic estimate of the missing data. Daily Tmax and Tmin values for these stations can be estimated with more than 99% confidence, except for Chamberlain COOP and Oacoma AWDN minimum temperatures where estimates can be made with more than 97.5% confidence. From the comparisons of the paired sites, a quality control diagnostic program can be created to flag when the differences between the sites are greater than 1 degree. These episodes can then be examined further to see if the differences were caused by weather events or equipment problems. Also the frequency of the occurrences could be evaluated to see if there might be some subtle equipment problems. Since these evaluations can be done remotely it could be an effective, economical and almost-continuous way to monitor the equipment between scheduled inspections and calibrations on site.

The $R^2$ values for the studied paired sites were above 0.97. This means that the temperature data could be integrated on a common finer resolution map of South Dakota maximum and minimum temperatures. With the two networks combined the most critical locations without weather stations could be identified for priority placement of future AWDN equipment.

In the future, hourly temperatures could be compared between the South Dakota AWDN sites and the nearest RAWS and airport sites. The purpose of this would be to verify if there were strong enough correlations to integrate the observations onto a common map.

In the future, studies should be done with rain gauges across South Dakota to establish statistical relationship between nearby stations.

Future research should be completed regarding whether the use of several observation stations would produce a better mathematical diagnostic model for evaluation of equipment rather than the closest. And research should be done to see if making seasonal or monthly models would improve estimates for replacing missing data over the annual model studied. Such high correlations as found in this study indicate that data from one station type can be used to estimate data for the other station type. Quality control and missing data estimation are essential to maintaining weather data archives. Knowing the variations and biases between two or more weather stations that are in close proximity, assists in developing and performing quality control procedures. Computer models could be created to estimate and substitute missing weather data from surrounding stations, and these models would automate the task of quality control on a near real-time basis.
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**LITERATURE CITED**


PLASTIC MULCH COLOR EFFECTS ON ECHINACEA GROWTH, SURVIVAL, AND ROOT PHENOLIC MARKER COMPOUNDS

R.L. Burrows  
Department of Horticulture, Forestry, Landscape and Parks

R.N. Reese  
Department of Biology and Microbiology  
South Dakota State University  
Brookings, SD 57007

ABSTRACT

Colored plastic mulches have been shown to affect growth as well as chemical composition of a number of herbaceous plant species. We tested the effects of blue, dark green (IRT), and red plastic mulches on growth of Echinacea angustifolia in a three-year field experiment. Plant survival in the second and third years was significantly higher in the red-mulch treatment compared to the other two colors. There were no significant detectable treatment effects on flower number, leafspot disease incidence, shoot weight, root dry weight, or root phenolic composition.

Keywords

Medicinal herb, coneflower, plasticulture

INTRODUCTION

Many herbaceous plant species have been shown to respond to various colors of plastic mulches with differences in plant growth, form, yield, or even chemical content. For example, basil (Ocimum basilicum) grown over yellow and green surfaces produced significantly higher concentrations of aroma compounds and phenolics compared to white, red, black, or blue surfaces. In the same experiment, basil leaves grown over red surfaces had greater area and fresh weight than those developing over black surfaces (Loughrin, 2001). Kasperbauer, et. al. (2001) found that strawberries had different concentrations of aroma compounds and organic acids when grown over red vs. black mulches. Carrot root phenolic concentrations were highest under yellow- or black-covered plots compared to those grown with red, white, blue, or green soil covers, while roots from yellow- and white-covered plots had highest concentrations of b-carotene and ascorbic acid (Antonius and Kasperbauer, 2002). Echinacea angustifolia showed...
higher second year plant weights when grown on black plastic rather than white or on bare soil in the mountains of North Carolina (Davis and Cox, 2004).

Our objective was to test whether different colored mulches would affect production of medicinal components of *E. angustifolia*, as measured by total phenolic concentration. We also monitored effects of the mulch color on other plant growth parameters, including survival.

**MATERIALS AND METHODS**

**Transplants.** Plant material was from two sources: seedlings from locally collected seed, and root cuttings from six mature plants that had been started from seed. The cuttings and seedling plants were started and grown in the greenhouse for eight and twelve weeks, respectively, hardened off for one week, and planted in the field plots in early July, 2002.

**Experimental design.** The research was carried out at the N.E. Hansen research farm near Brookings, South Dakota. Soil type is Vienna Brookings Complex, silty loam with a pH of 7.6, and organic matter 3.1%. Plots were laid out in a randomized complete block design, with four blocks. The three treatments (blue, dark green (IRT), or red plastic mulch) were applied to 3.2 m x 1 m plots, separated by 1.5 m between plots. Each plot was planted with 9 plants spaced 30 cm apart; there were five seedlings and four clonal plants in each plot. Drip irrigation was laid under the mulch and used only during plant establishment.
The plots were maintained for three years. Survival and flowering data was collected in early summer and fall of 2003 and 2004, leafspot intensity was visually rated on a 0-5 scale in July 2004, and plants were harvested in Sept. 2004. Whole plants were dug to a depth of 30 cm., which included the majority of the roots, and placed into paper bags for further processing. After recording stem and flower numbers, the shoots were separated from roots, dried, and weighed. Roots were gently washed to remove adhering soil, air-dried for 14 days, and weighed prior to grinding for further analyses. Total phenolic concentrations were spectrophotometrically determined on acetone/water/acetic acid extracts of the ground Echinacea roots using the Folin-Ciocalteu method of Slinkard and Singleton (1977). Phenolic concentrations were expressed as gallic acid equivalents.

Results were analyzed by analysis of variance (MSUSTAT) and means separated by Fisher’s Protected Least Significant Difference at p=0.05. Chi-square analyses were used to test for differences across treatments by clone, and Pearson correlations were used to examine relationships between phenolic content and other variables across and within treatments.

RESULTS AND CONCLUSIONS

Plant survival in the second and third years was significantly higher in the plots with red mulch compared to the IRT (dark green) and blue mulches (Fig. 1). We observed no significant treatment differences in early or late-season flower numbers either year, or in leafspot intensity (data not shown). Although root dry weights of surviving plants (Fig. 2) followed the same trend as the plant survival,
differences were not statistically significant. There were also no detectable significant treatment differences in other harvested plant data: stem and flower number, shoot weight, or total phenolic content of the roots, whether across all plants or within clones or treatments (data not shown). Leafspot was negatively correlated ($r=-0.335; p=0.006$) with root dry weight, suggesting that this disease can significantly impact root yields, and control measures should be considered by growers.

From our results, it appears that growers should consider using the red mulch, as it gave the highest plant survival, and there is no evidence it impacted phenolic concentrations differently than other mulch colors.

REFERENCES


SSR MARKERS AND SOYBEAN APHID RESISTANCE IN A CAGED FIELD TRIAL OF F$_3$-DERIVED SOYBEAN LINES

Department of Plant Science
South Dakota State University
Brookings SD 57007

ABSTRACT

The soybean aphid has recently become an economically important pest of soybean in South Dakota and the surrounding region. Aphid resistance and associated molecular markers have been identified in the cultivar Dowling and other lines. Our objective in this work was to confirm the utility of simple sequence repeat (SSR) markers that can be used to assist in introgressing the aphid resistance gene \textit{Rag1} from the cultivar Dowling into South Dakota soybeans. F$_3$-derived lines from selected F$_3$ plants from two populations of the 3-way cross (SDX00R-039-42XPI71506)$\times$Dowling and one population of the 3-way cross (SD01-5RXPI71506)$\times$Dowling were evaluated for resistance to soybean aphid in a field cage trial in 2006, and SSR markers flanking the \textit{Rag1} gene on the M linkage group were characterized in each line. Satt540 and Satt245 showed the segregation ratios expected for these linked loci, and aphid resistance was significantly associated with the Dowling alleles at these SSR markers.

INTRODUCTION

Genes for resistance to soybean aphid from the cultivars Dowling and Jackson have been localized to linkage group M, and associated markers have been described (Hill et al. 2004, 2005; Li et al. 2007). Additional sources of aphid resistance have also been identified in the soybean germplasm (Diaz-Montano et al. 2006; Mensah et al. 2005; Hesler et al. 2007). QTLs associated with resistance to other insects have also been localized to M and other linkage groups (Narvel et al. 2001; Terry et al. 2000; Walker et al. 2004; Zhu et al. 2006).

The objectives of the work described here were to identify lines with aphid resistance from Dowling as well as potential aphid resistance from PI71506, to confirm the utility of the simple sequence repeat (SSR) markers identified by Li et al. (2007) for selection of \textit{Rag1} gene resistance, and to determine whether aphid resistance from PI71506 was associated with the \textit{Rag1} gene or other genetic loci.
MATERIALS AND METHODS

**Plant material.** There were three 3-way cross populations, produced from crosses among 4 lines. The lines used were two SD soybean breeding lines, SDX00R-039-42 and SD01-5R; a Plant Introduction (PI) line, PI71506; and the aphid resistant variety Dowling (Hill et al. 2005). Crosses of SDX00R-039-42 X PI71506 and SD01-5R X PI71506 were made in summer 2004 in the field. The F₁ seeds were planted in the greenhouse during winter 2004-2005, and the resulting F₁ plants were crossed to Dowling. The resulting 3-way F₁ plants were selfed to produce 3-way cross F₂ plants. These were grown in the greenhouse in winter 2005-06 and bioassayed for aphid resistance in controlled greenhouse infestations. There were 3 F₂ plants on which aphids did not survive: 2 plants from the cross (SDX00R-039-42XPI71506)XDowling, and one plant from the cross (SD01-5RXPI71506)XDowling. Seeds were harvested from these 3 selected plants, and the resulting F₃ families were grown in the greenhouse in winter 2005-06, and bioassayed for aphid antibiosis in the greenhouse and for aphid antixenosis by lab assays. Plants that did not support aphid survival and which were not preferred by aphids were selected, and seed from these plants were composited as F₃-derived lines, which were grown in the following caged aphid trial in summer 2006.

**Caged field trial.** The F₃-derived lines were grown in 3-foot rows in a 64' x 42' cage that was designed to keep aphids in and exclude predators, in the field at the SDAES research farm near Aurora SD. Each F₃-derived family was grown as a separate block. The F₃-derived lines from the first and second plants derived from SDX00R-039-42XPI71506)XDowling were grown as Families 1 and 2 in blocks 1 and 2, respectively. Family 3, comprising the F₃-derived lines from the cross (SD01-5RXPI71506)XDowling, was planted in block 3. Each block also included the control lines, which included the parents if available, another susceptible line (519-R5, Syngenta), and resistant lines with the *Rag1* gene introgressed from Dowling. Each line was grown in a 3-foot un-replicated row.

Five aphids (collected from a field near Brookings SD in 2006 and maintained in the greenhouse on susceptible soybeans) were placed on every plant in each row, on 11 July 2006. Aphids were counted on 2, 8, 16, and 23 August 2006, on 5 plants in each row, including the first tagged plant, and 4 other randomly selected plants (not necessarily tagged plants). Plant growth stage was recorded at each counting.

**SSR markers.** In each row, 4 plants were randomly selected and tagged for DNA samples. Unexpanded leaflets (up to 1 cm in length) were collected from each of these plants and pressed onto FTA cards (Whatman Inc.) for DNA extraction. DNA was extracted according to the manufacturer’s protocol, and amplified by PCR in 25 µl reaction volumes, using 1.5 mM MgCl₂, 0.200 mM of each dNTP (Sigma), 2 µM of each primer (IDT), in 1X reaction buffer, with 0.625 to 1.5 units Taq polymerase (New England Biolabs). SSR markers included Satt435 and/or Satt540, Satt463 and/or Satt245, Satt536, and Satt250. Primer sequences were obtained from SoyBase. PCR amplifications were conducted with an initial denaturation at 94 or 95 C for 2 min, then 40 cycles of
92 C for 30 sec, 45 C to 50 C for 30 to 40 sec, and 68 C for 30 to 60 sec, with a final extension at 68-72 C for 2 to 7 min. The reaction products were separated by electrophoresis on 3.75% or 4.5% agarose gels, at 90 to 137 V, on ice, for 3 to 9 hours.

SSR genotypes were characterized for each of the 4 tagged plants per plot. In each plot, aphids were counted on the first tagged plant and on 4 other plants randomly chosen on each counting date. Data were analyzed by general linear model (GLM) analysis of variance (SAS Institute, 1982). Where aphid counts were made on tagged plants of known genotype, data were analyzed on an individual plant genotype basis. For analysis of aphid numbers in whole plots, we used the combined allele composition of all 4 plants characterized within a plot as the plot (line) genotype. Mean aphid numbers per plot were then analyzed in relation to the plot/line genotype, which was thus composed of 8 possible alleles per SSR marker.

RESULTS

Aphid numbers varied significantly depending on the date on which they were counted (p<0.0001), block (p<0.0001), the line evaluated (p<0.0001), and interactions among these effects (R²=0.86 for the model, p<0.0001).

There were 9 parent or control lines in this trial. These reference lines were known to be resistant or susceptible to the soybean aphid, and were analyzed separately to confirm whether the expected differences in aphid resistance were observed. The model was significant (R²=0.87, p<0.0001), with variation attributable to count date, block, line and interactions highly significant (p<0.0001 each). However, differences among lines diminished by mid-August. Variation attributable to lines accounted for 44% of the variation in aphid numbers on August 2, and 53% on August 8. By August 16, the R² for lines had diminished to 0.16; and by August 23, the model was not significant.

SSR markers flanking the R gene were chosen based on proximity and detectable polymorphism. Satt435 (M_38.94 cM) and Satt463 (M_50.097 cM), designated as selectable markers for the Dowling Rag1 gene by Hill et al. (2005), showed little variation in allele size between some of the parent lines used in these crosses, so the flanking markers Satt540 (M_35.85) and Satt245 (M_53.54) were used instead for the SDX005-039-42 families, and Satt540 and Satt435 were used for the SD01-5R family. Satt435 and Satt463 are approximately 11 cM apart; Satt540 and Satt245 are approximately 18 cM apart. Allele sizes for the the SSR markers Satt540 and Satt245 in parent and control lines are given in Table 1.

In general, the susceptible control lines 519-R5 (Syngenta) and SD01-76R supported significantly higher numbers of aphids than the resistant control lines, e.g., the LD05 and LDXG series, and PI71506 (Table 1). However, by August 16, all lines had an average of over 500 aphids per plant.

The change in aphid populations over time differed among parent and control lines (Fig 1), with the fastest increase in the susceptible lines (519-5R and SD01-76R), and delayed population buildup in the lines carrying the Rag1 gene.
### Table 1. SSR genotypes and mean number of aphids per plant, of parent and control lines, averaged over all count dates.

<table>
<thead>
<tr>
<th>Line</th>
<th>Genotype</th>
<th>Satt245</th>
<th>Satt540</th>
<th>Aphids</th>
</tr>
</thead>
<tbody>
<tr>
<td>519-R5 (Syngenta)</td>
<td></td>
<td>215215</td>
<td>145145</td>
<td>2,270 a</td>
</tr>
<tr>
<td>SDX00R-039-42</td>
<td></td>
<td>215215</td>
<td>145145</td>
<td>2,223 a</td>
</tr>
<tr>
<td>SD01-76R</td>
<td></td>
<td>215215</td>
<td>160160</td>
<td>2,223 a</td>
</tr>
<tr>
<td>SD01-5R</td>
<td></td>
<td>215215</td>
<td>160160</td>
<td></td>
</tr>
<tr>
<td>LD05-16106</td>
<td></td>
<td>195195</td>
<td>164164</td>
<td>668 b</td>
</tr>
<tr>
<td>LDXG-1</td>
<td></td>
<td>195215</td>
<td>150175</td>
<td>663 b</td>
</tr>
<tr>
<td>LDXG-3</td>
<td></td>
<td>195215</td>
<td>164164</td>
<td>375 bc</td>
</tr>
<tr>
<td>LD05-16094</td>
<td></td>
<td>195195</td>
<td>164164</td>
<td>339 bc</td>
</tr>
<tr>
<td>LD05-16066</td>
<td></td>
<td>195195</td>
<td>164164</td>
<td>280 c</td>
</tr>
<tr>
<td>PI71506</td>
<td></td>
<td>215215</td>
<td>155155</td>
<td>221 c</td>
</tr>
<tr>
<td>LD05-16143</td>
<td></td>
<td>195195</td>
<td>164164</td>
<td>124 c</td>
</tr>
<tr>
<td>Dowling</td>
<td></td>
<td>195195</td>
<td>164164</td>
<td></td>
</tr>
</tbody>
</table>

1Means followed by the same letter do not differ by Student Newman-Keuls test, \(p<0.05\)

![Figure 1. Mean number of aphids on parent and control lines over time.](image-url)
from Dowling. PI71506 displayed a similarly slow rate of increase in aphids initially, but reached aphid numbers equivalent to those of the susceptible lines by August 16. By August 23 there were no differences in aphid numbers among these lines.

Previous analyses had shown that each of the individual 3-way F_2 parent plants (of the F_3-derived lines) was heterozygous for both of the flanking SSR markers. Therefore, the F_3s and F_3-derived lines were expected to segregate for these markers also.

The date when aphids were counted and family (block) effects were significant in the overall analysis of F_3-derived lines. There were fewer aphids in Family 2 throughout the trial (Fig 2). Each F_3-derived family comprised different lines, and the SSR allele composition and effects differed among families, which were therefore analyzed separately. Within each family, the number of aphids differed significantly depending on the line, date counted, and their interaction. Representative aphid means by line within each family on August 8 (the time at which variation among lines was most significant) are given in Table 2.

On tagged plants whose individual genotype was determined for the Rag1-flanking SSR markers, aphid numbers varied in relation to the date counted, Satt540 alleles, and Satt245 alleles (p<0.0017, R^2=0.248). Aphid numbers were up to 3 times higher on plants with the susceptible alleles for both Satt540 and Satt245 than on plants that were homozygous for the Dowling Rag1 gene alleles at both flanking markers (Table 3). Plants with the Dowling parental genotype at both SSR loci supported significantly fewer aphids than plants with either of the other parental genotypes, or that were heterozygous for one or both SSR markers (Table 4). The combined heterozygotes were intermediate in Family 1, i.e., dominance did not appear to be complete at the Rag1 locus.

![Figure 2. Mean number of aphids in the 3 families of F_3-derived lines, over time.](image-url)
Table 2. Mean number of aphids counted August 8 on representative lines from Family 1 ((SDX00R-039-42XPI71506)XDowling), Family 2 ((SDX00R-039-42XPI71506)XDowling), and Family 3 ((SD01-5RXPI71506)XDowling). Family 1: $p_{model}<0.0001$, $R^2=0.88$; Family 2: $p_{model}=0.0001$, $R^2=0.87$; Family 3: $p_{model}=0.0001$, $R^2=0.95$.

<table>
<thead>
<tr>
<th>FAMILY 1</th>
<th>FAMILY 2</th>
<th>FAMILY 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line</td>
<td>Aphids(^a)</td>
<td>Line</td>
</tr>
<tr>
<td>SDX04R AP 1-31</td>
<td>3,700 a</td>
<td>SDX04R AP 2-10</td>
</tr>
<tr>
<td>SDX04R AP 1-25</td>
<td>3,500 ab</td>
<td>SDX04R AP 2-18</td>
</tr>
<tr>
<td>SDX04R AP 1-67</td>
<td>3,200 abc</td>
<td>SDX04R AP 2-32</td>
</tr>
<tr>
<td>SDX04R AP 1-74</td>
<td>2,500 cd</td>
<td>SDX04R AP 2-38</td>
</tr>
<tr>
<td>SDX04R AP 1-35</td>
<td>1,912 def</td>
<td>SDX04R AP 2-20</td>
</tr>
<tr>
<td>SDX04R AP 1-68</td>
<td>1,400 efgh</td>
<td>SDX04R AP 2-36</td>
</tr>
<tr>
<td>SDX04R AP 1-49</td>
<td>1,000 fgghi</td>
<td>SDX04R AP 2-30</td>
</tr>
<tr>
<td>SDX04R AP 1-78</td>
<td>785 ghi</td>
<td>SDX04R AP 2-26</td>
</tr>
<tr>
<td>SDX04R AP 1-28</td>
<td>584 ghij</td>
<td>SDX04R AP 2-27</td>
</tr>
<tr>
<td>SDX04R AP 1-05</td>
<td>331 ghi</td>
<td>SDX04R AP 2-23</td>
</tr>
<tr>
<td>SDX04R AP 1-73</td>
<td>179 h</td>
<td>SDX04R AP 2-21</td>
</tr>
<tr>
<td>SDX04R AP 1-76</td>
<td>92 i</td>
<td>SDX04R AP 2-01</td>
</tr>
<tr>
<td>SDX04R AP 1-82</td>
<td>39 i</td>
<td></td>
</tr>
<tr>
<td>SDX04R AP 1-83</td>
<td>9 i</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Means followed by the same letter, within each column, do not differ by Student Newman-Keuls test, $p<0.05$.

Table 3. Individual [tagged] plants of Family 1 F\(_3\)-derived lines. Mean number of aphids (averaged over all countdates) per genotypic class of Satt245 and Satt540 SSR markers flanking the Rag1 gene.

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>Satt540</th>
<th>Satt245</th>
<th>Number of Plants</th>
<th>Aphids</th>
</tr>
</thead>
<tbody>
<tr>
<td>164 164</td>
<td>195 195</td>
<td>14</td>
<td>397 a</td>
<td></td>
</tr>
<tr>
<td>164 164</td>
<td>195 215</td>
<td>7</td>
<td>407 a</td>
<td></td>
</tr>
<tr>
<td>164 164</td>
<td>215 215</td>
<td>4</td>
<td>588 ab</td>
<td></td>
</tr>
<tr>
<td>164 145</td>
<td>195 215</td>
<td>6</td>
<td>635 ab</td>
<td></td>
</tr>
<tr>
<td>145 145</td>
<td>195 215</td>
<td>4</td>
<td>670 ab</td>
<td></td>
</tr>
<tr>
<td>164 145</td>
<td>195 195</td>
<td>7</td>
<td>682 ab</td>
<td></td>
</tr>
<tr>
<td>145 145</td>
<td>195 195</td>
<td>3</td>
<td>940 ab</td>
<td></td>
</tr>
<tr>
<td>145 145</td>
<td>215 215</td>
<td>24</td>
<td>960 ab</td>
<td></td>
</tr>
<tr>
<td>164 145</td>
<td>215 215</td>
<td>3</td>
<td>1,304 b</td>
<td></td>
</tr>
</tbody>
</table>

\(^b\)Means followed by the same letter do not differ by Student-Newman-Keuls test, $p<0.05$. 


Line genotypes were determined from characterization of flanking marker alleles of 4 plants per plot (line). Both of the \textit{Rag1}-flanking SSR markers, Satt245 and Satt540, were significantly associated with variation in aphid numbers (p<0.001) in Families 1 and 2, and Satt245 was significant in Family 3.

In Family 1, segregation of the F$_3$-derived lines did not differ significantly from that expected assuming the parent was heterozygous and Satt540 and Satt245 are linked at a distance of ca. 18 cM ($X^2=13.35$, p>0.10) (Table 5). In Family 1, the Satt540 and Satt245 alleles corresponded to those of Dowling and the SDX00R-039-42 parent; alleles from PI 71506 were not observed in this family. Aphid numbers differed significantly among genotypes and date counted. Lines that were homozygous for both the 195 bp (Dowling) allele of Satt245 and the 164 bp (Dowling) allele of Satt540 had fewer aphids than other lines. Aphid means by genotypic class for the combined \textit{Rag1} flanking markers, Satt540 and Satt245, over the length of the trial, are shown in Fig 3.

### Table 4. Individual plants [tagged] of Family 1 and Family 2 F$_3$-derived lines. Least squares mean number of aphids (adjusted for change over dates counted) per parental and combined heterozygous genotypes.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>FAMILY 1</th>
<th>FAMILY 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Plants</td>
<td>Number of Plats</td>
<td>Aphids$^2$</td>
</tr>
<tr>
<td>Dowling</td>
<td>14</td>
<td>20</td>
<td>398 a</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>34</td>
<td>51</td>
<td>643 b</td>
</tr>
<tr>
<td>SDX00R-039-42</td>
<td>24</td>
<td>11</td>
<td>971 c</td>
</tr>
</tbody>
</table>

$^2$Means followed by the same letter do not differ by LSmeans test.

### Table 5. Segregation of Satt540 and Satt245 alleles in Family 1 F$_3$-derived lines.

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>Satt540</th>
<th>Satt245</th>
<th>Number of Lines(O)</th>
<th>Expected (E)</th>
<th>(O-E)$^2$/E</th>
</tr>
</thead>
<tbody>
<tr>
<td>164 164</td>
<td>195 195</td>
<td></td>
<td>8</td>
<td>(0.168) 12</td>
<td>1.30</td>
</tr>
<tr>
<td>164 164</td>
<td>195 215</td>
<td></td>
<td>4</td>
<td>(0.037) 3</td>
<td>0.33</td>
</tr>
<tr>
<td>164 164</td>
<td>215 215</td>
<td></td>
<td>0</td>
<td>(0.008) 1</td>
<td>1.00</td>
</tr>
<tr>
<td>164 145</td>
<td>195 195</td>
<td></td>
<td>5</td>
<td>(0.074) 5</td>
<td>0</td>
</tr>
<tr>
<td>164 145</td>
<td>195 215</td>
<td></td>
<td>37</td>
<td>(0.352) 25</td>
<td>5.76</td>
</tr>
<tr>
<td>164 145</td>
<td>215 215</td>
<td></td>
<td>7</td>
<td>(0.074) 5</td>
<td>0.80</td>
</tr>
<tr>
<td>145 145</td>
<td>195 195</td>
<td></td>
<td>0</td>
<td>(0.008) 1</td>
<td>1.00</td>
</tr>
<tr>
<td>145 145</td>
<td>195 215</td>
<td></td>
<td>2</td>
<td>(0.074) 5</td>
<td>1.80</td>
</tr>
<tr>
<td>145 145</td>
<td>215 215</td>
<td></td>
<td>8</td>
<td>(0.168) 12</td>
<td>1.33</td>
</tr>
</tbody>
</table>

$^2X^2$ 71

$^2X^2$ 13.32 (ns, p>0.10)
Family 2 also segregated for alleles of Satt245 and Satt540, and did not deviate from the expected linkage between these markers (Table 6). The allele composition of this family differed from that of the Family 1, i.e., the Satt245 and Satt540 alleles from Dowling and PI71506 were present in Family 2, and the corresponding alleles from SDX00R-039-42 were not. Aphid numbers were significantly lower in lines that carried both the Satt245 195 bp allele (from Dowling) and the Satt540 164 bp allele (from Dowling). Aphid means at each date counted, by genotypic class for the combined Rag1 flanking markers, Satt540 and Satt245, are shown in Fig 4.

Table 6. Family 2 F$_3$-derived lines. Segregation of Satt540 and Satt245 genotypic classes.

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>Number of Lines (O)</th>
<th>Expected (E)</th>
<th>(O-E)$^2$/E</th>
</tr>
</thead>
<tbody>
<tr>
<td>164 164</td>
<td>195 195</td>
<td>5</td>
<td>(0.168) 4.7</td>
</tr>
<tr>
<td>164 164</td>
<td>195 213</td>
<td>2</td>
<td>(0.037) 1.0</td>
</tr>
<tr>
<td>164 164</td>
<td>213 213</td>
<td>0</td>
<td>(0.008) 0.2</td>
</tr>
<tr>
<td>164 155</td>
<td>195 195</td>
<td>2</td>
<td>(0.074) 2.1</td>
</tr>
<tr>
<td>164 155</td>
<td>195 213</td>
<td>9</td>
<td>(0.352) 9.9</td>
</tr>
<tr>
<td>164 155</td>
<td>213 213</td>
<td>2</td>
<td>(0.074) 2.1</td>
</tr>
<tr>
<td>155 155</td>
<td>195 195</td>
<td>1</td>
<td>(0.008) 0.2</td>
</tr>
<tr>
<td>155 155</td>
<td>195 213</td>
<td>3</td>
<td>(0.074) 2.1</td>
</tr>
<tr>
<td>155 155</td>
<td>213 213</td>
<td>3</td>
<td>(0.168) 4.7</td>
</tr>
</tbody>
</table>

\[ X^2 \] 
5.86 (ns, p>0.50)
In Family 3, Satt245 alleles segregated 1:2:1 for the Dowling: heterozygotes: SD01-SR alleles (\(X^2=0.125, p>0.90\)), and aphid numbers varied significantly depending on Satt245 genotype until August 23. Aphid numbers were over 4 times higher on plants with the SDX00R-039-42 genotype than on those with the Dowling alleles for Satt245. We were unable to reliably and consistently determine the alleles for either Satt540 or Satt435 in many of the plants in the F3-derived lines, due to lack of resolution of the small differences among the allele variants, and apparent interference from some of the extracts, as well as sample degradation.

Aphid numbers significantly decreased in relation to the number of 164 bp (Dowling) alleles for Satt540, and to a lesser extent the number of 195 bp (Dowling) alleles for Satt245, present in Family 1 throughout the trial. In Family 2, aphid numbers also declined with increasing numbers of both 164 bp alleles for Satt540 and 195 bp alleles for Satt245.

Aphid numbers differed significantly among allele classes for each of the individual SSR loci flanking the \(Rag1\) gene. For example, in Family 1, the presence of 2 copies of the 195 bp (Dowling) allele of Satt245 reduced aphid numbers (averaged over the entire trial) to less than 50% of the number of aphids on plants that were homozygous for the 215 bp allele of Satt245, and the presence of 1 copy of the 195 bp allele reduced aphid numbers by about one-third (\(p_{\text{model}}<0.0001, R^2=0.22\)).

The reliability of Satt540 and Satt245 alleles as predictors of resistance or susceptibility in these lines was evaluated by characterizing alleles in each line, and comparing the predicted response to actual aphid counts. A line was characterized as susceptible if all plants in the line were homozygous for the susceptible parent alleles at both Satt540 and Satt245, and as resistant if all plants within the line were homozygous for the Dowling alleles at both Satt540 and Satt245.

Using this method, 10 lines in Block 1 were characterized as susceptible. On August 8, 90% of these lines had over 500 aphids per plant. Also in Block 1, 9

![Figure 4. Mean number of aphids per plant by SSR genotypic class of F3-derived lines of Family 2.](PI71506)
lines were predicted to be resistant, and 78% of these had less than 300 aphids per plant on August 8. In Block 2, 4 lines were predicted to be susceptible; however, 3 out of these 4 plants had less than 300 aphids per plant on August 8. Six plants in Block 2 were characterized as resistant, and none of these had more than 300 aphids per plant on August 8.

In Block 3, only Satt245 alleles were available to predict aphid resistance. Of 12 lines characterized as susceptible, 2 (17%) had less than 500 aphids per plant on August 8. Of 13 lines identified as resistant, 5 (38%) had over 500 aphids per plant on August 8.

**DISCUSSION**

For a given locus (diploid), a single progeny plant resulting from a 3-way cross can be expected to carry one allele from the third parent plus one allele from either the first parent or the second parent, but not both \([(a'a' X a'a') X a'a'] = \frac{1}{2} (a'a') + \frac{1}{2} (a'a')\). This was observed in the 2 families derived from the 3-way cross with SDX00R-039-42, each of which was derived from a single individual plant. The \(Rag1\)-flanking SSR marker allele composition of Family 1 differed from that of Family 2, reflecting the probability that the original F\(_1\) parents of the 3-way cross differed, in that although both carried the Dowling alleles (\(a'\) in the previous example) for Satt540 and Satt245, one carried as well the Satt540 and Satt245 alleles from the susceptible parent (SDX00R-039-42), and the other carried the Satt540 and Satt245 alleles from PI 71506.

In both families, aphid resistance was associated with the Dowling alleles of the SSR markers flanking the \(Rag1\) gene. Both of the Dowling alleles for Satt540 and Satt245 were required in homozygous condition for optimum aphid resistance in these lines, suggesting that in our populations, \(Rag1\) resistance was not completely dominant. It also appears that in these lines, aphid numbers may overcome the \(Rag1\)-associated resistance within a few weeks.

The Satt540 and Satt245 alleles from PI71506 were present in Family 2 and appeared to be associated with susceptibility to aphids. However, Family 2 generally supported fewer aphids than Family 1; thus, other genes or QTLs that may be associated with the M linkage group or perhaps elsewhere in the genome may contribute to aphid resistance or non-preference. Narvel et al. (2001) have identified a major insect-resistance QTL in the M linkage group, south of the \(Rag1\) gene. However, our analyses of one marker, Satt536, in the region of this QTL displayed little or no polymorphism among Dowling, PI71506, and SDX00039-42.

In conclusion, aphid resistance derived from the \(Rag1\) gene of Dowling can be selected using the flanking markers Satt540 and Satt245. However, this resistance may not always be dominant in some populations; and resistance may not hold up for extended periods or under heavy aphid infestation. Aphid resistance that may be derived from PI71506 does not appear to be associated with the Satt540 to Satt245 region.
LITERATURE CITED


SOUTH DAKOTA’S NATURAL HISTORY COLLECTIONS: AN ENDANGERED TEACHING AND RESEARCH RESOURCE

M. L. Gabel
Department of Biology
Black Hills State University
Spearfish, SD 57799

P. J. Johnson
Plant Science Department
South Dakota State University
Brookings, SD 57007

G.E. Larson
Biology and Microbiology
South Dakota State University
Brookings, SD 57007

D. J. Ode
South Dakota Natural Heritage Program
Department of Game, Fish and Parks
Pierre, SD 57501

H. A. Downing
College of Arts and Science
Black Hills State University
Spearfish, SD 57799

G. M. Kostel
Department of Biology
Black Hills State University
Spearfish, SD 57799

ABSTRACT

Natural history collections including plants, fungi, insects and vertebrates are the critical base for research and teaching in the biological sciences. There are several important and irreplaceable natural history collections associated with the institutions of higher education in South Dakota that are the result of over a century of work by biologists in the state. Unfortunately, at the present time there are looming dangers to these collections including lack of staffing, and constant threats due to insects, fungi, and unstable environmental conditions. It is critical to provide support for these collections to ensure the preservation of valuable information in the collections, to allow future generations of South
Dakotans access to their natural heritage, to study biodiversity, to support basic and applied science, to plan for future endeavors, to monitor pest species, and to provide baseline data for future studies. Recommendations are made for support for collections in the university system.

IMPORTANCE AND USES OF NATURAL HISTORY COLLECTIONS

Natural history collections contain a wealth of data including genetic and phylogenetic information within the organisms, and ecological and biogeographical information on the specimen labels which yield a scientific specimen of great intrinsic value (Lane, 1996). Natural history collections are the acknowledged foundation of research in biological sciences. The information content of a natural history collection is tremendous. Biological specimens document: (a) the time of appearance or disappearance of an organism in a particular locality; (b) the range of variation within a species; (c) the nature of evolutionary processes; and (d) the life cycles of a particular organism. They also (e) provide material for study away from the field or during another season. They serve as: (f) voucher specimens, that document the identity of organisms used in taxonomic, chemical (i.e., DNA), or cytological, or other studies, and as (g) type specimens upon which names are based.

A few examples of their uses include the following:

- contribute “unique and invaluable insights to the study of pathogens, vectors of disease and environmental contaminants” (Suarez and Tsutsui, 2004),
- provide for the study of numerous environmental contaminants including mercury, DDT and atrazine (EPA, 2002; Hickey and Anderson, 1968; Hayes et al., 2002),
- document the pace and ecological consequences of biological change caused by habitat loss (Shaffer et al., 1998),
- document the effects of climate change on numerous organisms including changes in distribution and changes in the biology of particular species in response to climate change (Suarez and Tsutsui, 2004), and
- determine the distribution of invading species, identify the source of the invasion, and to gauge the ecological impact of the invaders (Suarez et al., 2001).

Scientific collections are obviously essential for taxonomic and systematic research, but collections also make significant contributions to basic and applied science, including anatomy, botany, ecology, genetics, morphology, mycology, phylogenetics, population biology, structural biology and zoology, by providing raw data and logistical support for these and other disciplines. In science, repeatability is a keystone of the process, and specimens used for scientific investigations must be vouchered in collections to ensure that species identifications can be confirmed and results interpreted correctly (Ruedas et al., 2000). Natural history collections are becoming increasingly important with new advances in biotechnology. It is now possible to use very small amounts of preserved mate-
rial for extraction and analysis of DNA to answer very important biological questions such as the origins and spread of Lyme disease (Persing et al., 1990). For these reasons professionally maintained natural history collections should be viewed as self-evidently essential to most biological investigations.

Because natural history collections play such an important role in societal endeavors, continuous physical and financial support is absolutely critical. Collections are most valuable in their original institutional and geographical context. Because they contain historical records linked to a time and place, lost collections cannot be replaced. Moreover, many populations of organisms documented in natural history collections no longer exist. Furthermore, some specimens cannot be replaced due to the imposition of constraints on collecting. Therefore institutions need to maintain their collections in perpetuity. Once an institution divests itself of a collection, the institution can never regain the benefits associated with the collections.

**IMPORTANCE OF NATURAL HISTORY COLLECTIONS TO SOUTH DAKOTA**

Natural history collections are important in understanding the history of our state and are critical for comparing modern and historical distributions of species by providing baseline data for future studies. These collections are also important in monitoring the presence of plant, fungal and insect pests in the state. Collections of biological organisms are present in all South Dakota universities, but size and importance of the collections varies widely. Some collections are larger and have significant research importance. Nearly all biology departments have small teaching collections of various organisms (Table 1).

South Dakota is one of the least biologically known areas in the United States (Great Plains Flora Association, 1986). Samson et al. (1998) reported that

<table>
<thead>
<tr>
<th>Institution</th>
<th>Type of collection</th>
<th>Number of specimens</th>
<th>Curator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Hills State University</td>
<td>Plants and Fungi</td>
<td>35,000</td>
<td>M. Gabel</td>
</tr>
<tr>
<td>South Dakota State University</td>
<td>Plants</td>
<td>45,000</td>
<td>G. Larson</td>
</tr>
<tr>
<td>South Dakota State University</td>
<td>Insects</td>
<td>1,200,000</td>
<td>P. Johnson</td>
</tr>
<tr>
<td>University of South Dakota</td>
<td>Plants</td>
<td>25,000</td>
<td>M. Nepokroeff</td>
</tr>
<tr>
<td><em><em>Smaller and Teaching</em> Collections in South Dakota</em>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dakota Wesleyan University</td>
<td>Plants</td>
<td>3,500</td>
<td></td>
</tr>
<tr>
<td>Northern State University</td>
<td>Plants</td>
<td>3,000</td>
<td></td>
</tr>
<tr>
<td>South Dakota State University</td>
<td>Fishes</td>
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</tr>
<tr>
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<td>Mammals</td>
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<td></td>
</tr>
<tr>
<td>South Dakota State University</td>
<td>Birds</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>W.H. Over Museum</td>
<td>Birds</td>
<td>1,700</td>
<td></td>
</tr>
</tbody>
</table>

* Note that nearly all institutions have small teaching collections including invertebrates, amphibians, reptiles, mammals, birds and plants not included in this table.
the Black Hills and surrounding grasslands in South Dakota and Wyoming are a priority landscape of biological significance with regard to biodiversity and ecological dynamics. Unfortunately, “basic floristic information is still lacking for …the grasslands of Montana, Wyoming, Colorado and the Dakotas” (Great Plains Flora Association, 1986).” The density of G1 to G3 species (G rankings indicate global rarity, e.g., G1 indicates that the species is found at five or fewer sites or is represented by 1000 or fewer individuals) in the Black Hills is 2.5 times greater than anywhere else in the Great Plains (Ostlie et al., 1997). Our natural history collections provide the primary information for understanding regional biotas and are thus the central critical elements in biodiversity and ecological studies.

Natural history collections are used widely to provide teaching specimens in South Dakota universities. While all universities have their own small teaching collections of major groups of plants and animals, teaching specimens are often augmented by research collections for instruction of undergraduate and graduate students.

The Severin-McDaniel Insect Research Collection (SMIRC) at South Dakota State University is estimated to contain approximately 1.2 million specimens and available rankings of collections place it as the largest in the northern Great Plains and one of the largest university-based arthropod collections in the United States. The collection is composed largely of insects and spiders from South Dakota and adjacent states, but approximately 15-20% of the specimens are from Colorado, Alaska, Yukon, Solomon Islands, Costa Rica and other national and international areas. The SMIRC also houses a small fossil collection and the historically important W. H. Over collection of mollusks that includes numerous specimens of freshwater clams that are extinct or near extinction in many river systems.

The Black Hills State University Herbarium is a rapidly growing facility with most collections from the Black Hills. The oldest specimens date to the 1870s, but the herbarium was not founded until the 1880s. In addition to approximately 35,000 vascular plant specimens, it is home to 10,000 plant fossils from the Great Plains and about 3000 fungal specimens including nearly all of the state record collections. At this writing the approximately 8,000 specimens of the Augustana College (Sioux Falls) Herbarium are being incorporated into the BHSU collection.

The South Dakota State University Herbarium (SDC) began at the inception of the institution in 1881, and indeed, the collection includes specimens collected in that year. The SDC includes an estimated 45,000 plant specimens emphasizing South Dakota and the Great Plains region. Professors Taylor, Gary E. Larson, and their students have been the principal collectors in recent times. Specimen trades with other herbaria in North America, including the University of Wyoming, Oregon State University, the University of Kansas and Northeast Louisiana State University, have expanded the geographic coverage of the herbarium.

Duplicate specimens from the herbarium are used in training students in botany courses that include Plant Systematics, Plant Ecology, Grasses and Grasslike Plants, Range Plant Identification, and Aquatic Plants. Many plant
samples are submitted for identification from personnel in the S.D. Cooperative Extension Service, federal agencies, and the public at large. Identifications of unknown plant samples are best verified by comparison with identified specimens in the herbarium. In this role, the herbarium is much like a reference library and it is used in the very same manner by graduate students and others conducting botanical field work in the region.

The University of South Dakota Herbarium houses about 25,000 specimens, and contains numerous historically important specimens collected by W. H. Over, S. S. Visher and A. C. McIntosh, workers important in the early botanical exploration of the state. The collection was the basis of the state flora (Van Bruggen, 1996), and the only South Dakota collection represented in the Flora of the Great Plains (Great Plains Flora Association, 1986). It is currently participating in the databasing project of plants from Western South Dakota and Eastern Wyoming.

ROLE OF THE CURATOR IN NATURAL HISTORY COLLECTIONS

A description of the job of the curator provided by the U.S. Department of Labor Bureau of Labor Statistics is as follows: “Curators administer the affairs of museums, zoos, aquariums, botanical gardens, nature centers, and historic sites.... Curators direct the acquisition, storage, and exhibition of collections, including negotiating and authorizing the purchase, sale, exchange, or loan of collections. They are also responsible for authenticating, evaluating, and categorizing the specimens in a collection. Curators oversee and help conduct the institution's research projects and related educational programs. Today, an increasing part of a curator’s duties involves fundraising and promotion, which may include the writing and reviewing of grant proposals, journal articles, and publicity materials, as well as attendance at meetings, conventions, and civic events” (http://www.bls.gov/oco/ocos065.htm).

Curatorial duties taken from a composite of job advertisements for curators in various disciplines include: strategic collection development, supporting departmental research programs, making collections available to a wide audience, management of loans, responding to inquiries for identifications and distributions, managing conservation procedures for specimens, maintaining a strong research program, database management, cataloging, various administrative duties, serving on committees, promoting the use of collections, participating in outreach and maintaining and expanding the collection.

The curator must arrange to have a significant library of specialized literature (floras, faunas, reprints, monographs) and electronic resources (CD-ROMs, Internet access) to operate a modern collection (Snow, 2005). Some of these resources cost several hundreds or thousands of dollars each and are very difficult to purchase on limited budgets.

A significant task not mentioned above is the role of the curator in informing the upper level academic administrators about the importance of the collection (Snow, 2005). Often administrators are not long-term employees of an
The more successful the collection becomes the more work that is required by the curator. It is sometimes difficult to convince administrators that a successful collection requires additional work (Snow, 2005).

**MONETARY VALUE OF COLLECTIONS**

A little known fact is that museums save both time and money. Natural history collections are “biological libraries” that are cost efficient repositories of accumulated knowledge and resources. Frequently, collections eliminate the need for expensive, time consuming and occasionally dangerous fieldwork because increasing costs of travel to distant locations cost the scientific community millions of dollars annually. Reducing the costs of studying vectors of disease, biological invasions and climate change provide direct financial and social benefits to society (Suarez and Tsutsui, 2004). Savings will increase as more collections are available online.

Various authors or groups have calculated the approximate value of natural history specimens. These calculations are typically based on the value of the collector’s time and the value of the curator and staff time, but the real monetary value of the specimens contained in these collections is difficult to ascertain, since historical specimens cannot be replaced. Recent discussions on a herbarium list server (herbaria@scarab.nacse.org) indicate that calculating the value of specimens is still an inexact exercise. Suggested value per specimen in collections ranged from $5 to $70. Commercial values for insects range in price from $2 to $15,000 per specimen (http://insectworld.com). Numerous attempts to evaluate collections of different taxa are documented in Nudds and Pettitt (1997).

Individual plant specimens have been valued from $10 (American Systematics Collections) to $52.50 (Armstrong, 1992). Insect specimens have been estimated by the South African Museum to cost about $22 (U.S. dollars) per replacement specimen (http://www.museums.org.za/sami/muse/entman/b_intro.html). Mammal specimens have been estimated to cost $43 (U.S. dollars) from field collection to incorporation in a research collection (Lee et al., 1982). The University of New Mexico natural history collections are insured by the State of New Mexico Risk Management Office. The collections in the herbarium are valued at $10 per specimen. This amount will not allow replacement of the irreplaceable historical specimens but the collection managers could partially re-build the collection if disaster were to strike. Whatever the figure, none take into account the intrinsic scientific value of the specimen. Since the collections are irreplaceable setting an absolute value per specimen is pointless since replacement is impossible.

Natural history collections are sometimes easy targets for administrators during budget crises (Gropp, 2003). Suarez and Tsutsui (2004) have argued that the housing and maintenance of natural history collections is inexpensive when compared to the potential costs of their absence. As an example, Mann (1997) described the costs of a “typical” foreign trip for obtaining specimens which included salaries and modest travel and in-country expenses totaling about $63,000. Raven (2003) asked how a state could beneficially use or preserve its natural capital if there are no institutions in which its diversity is documented.
None of the natural history collections in South Dakota have ever had designated staff positions. There is an urgent need to implement measures to protect the specimens from insect and fungal pests to ensure the very survival of these collections for posterity. Increasing demands of teaching, research and service on the curators of herbaria and animal collections in the state have resulted in less time for management and improvement of research collections.

Current curators of natural history collections in South Dakota are aging. The senior author (Gabel) who is at BHSU retired in 2003, but is still working in the herbarium as a volunteer. It is not known how long he can continue to fill this role. The person hired to fill Gabel’s teaching responsibilities does not have herbarium experience, nor does he have the time to work in the herbarium. Larson at the SDSU Herbarium has been curator for 27 years and is approaching retirement age. It is unknown what qualifications his replacement will have, or if the replacement will have the time or knowledge to manage the herbarium. Johnson at SDSU has an overwhelming responsibility to maintain the burgeoning insect collection as well as continue his other duties.

The University of South Dakota Herbarium was actively curated by T. Van Bruggen until 1988. At that time a replacement for Dr. Van Bruggen was hired who did not expend sufficient time or effort with the collection. At least 500 specimens from the collection were damaged by pest insects and discarded, including about 100 specimens of rare “lower” vascular plants on loan from BHSU to USD that were damaged severely. That person has since left but the damage to the collection remains a stark warning of the vulnerability of South Dakota natural history collections. Molly Nepokroeff at USD, while interested in the herbarium, has a research program in molecular biology.

In addition to convincing administrators of the value of the collections, it is incumbent upon natural history science staff to convince colleagues of the importance of the collections. When curators retire it is critical that they be replaced with qualified scientists.

Museums and universities must bear the cost of operations of their collections including maintaining databases and keeping the original specimens in good condition (Pennisi, 2005). The National Science Foundation (NSF) Biological Research Collections program limits awards to one-time support of specific goals or projects, with about half of the $4.5 million budget used for long-term digital data collection (Pennisi, 2005). Thus, though a good source for major renovation funds, the NSF is not a reliable source of operational support.

DATABASES AND NATURAL HISTORY COLLECTIONS

We have entered the Century of Biology (Carey, 1998). The demand for biological information is expected to increase exponentially to address issues such as invasive species, human health, sustainable development, biodiversity, endan-
gered species and environmental problems. The capacity to deliver information is one of the most important growth factors in any endeavor, and some natural history collections are poised to meet this demand.

The South Dakota Natural Heritage Database was established in 1981 and is currently funded by the Department of Game, Fish and Parks. The Natural Heritage Program is a member of NatureServe, an international network of biological inventories. The taxa represented in the database are generally those that are rare, threatened or endangered. Data searches are free to state agencies and available for a fee to others.

In South Dakota the only natural history collection currently web accessible is the grass database at Black Hills State University, with more than 10,000 specimen records from 15 herbaria, thanks to funding from the National Fish and Wildlife Foundation [https://www.bhsu.edu/artsandsciences/asfaculty/mgabel/herbariumdatabase/databaseIntroduction.html]. The National Science Foundation has recently awarded a grant to BHSU to increase BHSU Herbarium storage capacity and to construct a consolidated database of all vascular plant specimens for western South Dakota and eastern Wyoming. This database will include data from 16 herbaria including SDSU, USD, and numerous other collections. Web accessibility for the vascular plants of the study area will be available in 2009. Similar projects at the other collections in the state are at best in the proposal stages for grants and not explicitly supported by their institutions.

It is important that all specimens in the South Dakota natural history collections be incorporated into web accessible databases to fill gaps in natural history distributions (Card et al., 2005), and that the data from the state and region be present in international databases such as the Global Biodiversity Information Facility (GBIF). Web accessible databases are important to better understand the natural history of the state, to make baseline data available for various agencies including the South Dakota Wildlife Diversity program, [http://www.sdgfp.info/Wildlife/Diversity/index.htm], various regulatory agencies, land managers, pest control agencies, state researchers and researchers from around the world. Computing power has allowed the construction of databases of large amounts of information from natural history collections (Card et al., 2005). Web presence can be achieved relatively easily, but a far more valuable service for the scientific community (including faculty and students from South Dakota) and a much more prestigious web presence for databases can be achieved by participation in the GBIF ([http://www.gbif.org/]). This is a metadatabase of collections of specimens from around the world containing 91 million records from 168 providers (as of April, 2006).

Due to the heavy workload of the curatorial staff, the labor intensive nature of the work, and lack of financial support, it is unlikely given current staffing, that databasing will be completed without external funding. As such, South Dakota research and teaching institutions are not part of the global community of biology research, hence derelict in their public responsibility in managing natural resources. For example, the BHSU curator did not have time to write a fundable grant proposal until he retired and donated his time to the herbarium. It is critical that natural history collection curators have sufficient time to devote
to supervision of collections and to seek external funding for database development.

RECOMMENDATIONS TO ADMINISTRATORS REGARDING INSTITUTIONAL SUPPORT

Natural history collections “clearly embody the primary mission of higher education, which is the discovery and dissemination of knowledge” (Snow, 2005). They are also the original source of much of the knowledge we have about life on Earth. The emergence of molecular biology has not changed this fact, but has instead reinforced the importance of the collections.

Inactive natural history collections lose their value since species, genus, families and even higher order taxa are often renamed and realigned and need to be updated. Unattended specimens can become worthless as a result of damage from insects, fungi, heat, light or moisture. Specimens that are well-curated and well-preserved do increase in value with time (Snow, 2005). New digital technologies increase the value of specimens since data are more accessible to scientists.

Funding agencies such as the National Science Foundation (NSF) increasingly require evidence of continued institutional support for such facilities if grant applications are to be given serious consideration. NSF realizes that curation requires time and resources and these must be supplied internally (Pennisi, 2005). Snow (2005) reported several factors that are considered when reviewing grant proposals for collection activities including:

- Consistent, internally provided budgets for operations and maintenance are required (it is not realistic for curators to seek external funding to support routine operation).
- Proposals to NSF are highly competitive and require 6-10 weeks or more to complete.
- Staff in addition to the curator greatly expands the potential for activities.

Snow (2005) also noted that hiring a molecular taxonomist as a curator of a natural history collection may lead to neglect of the facility.

There are four large and irreplaceable natural history collections at universities in South Dakota, as well as several smaller valuable collections (Table 1). It is incumbent upon state institutions to provide operational support for natural history collections (Pennisi, 2005). Due to increased responsibilities in teaching, research and service it is not possible for the current curators to expand their duties to include proposals for large extramural grants without additional support. It is essential that the large collections, and secondarily the smaller collections be included in a modern web accessible database documenting the biodiversity of the state.

To this end we recommend that a minimum of 0.5 FTE be assigned to each of the four large collections (Table 1) by the Board of Regents. If, at some
later date an administrator should decide to give away a collection, the 0.5 FTE
would follow that collection, assuming it was given to another state university.
We are recommending that each institution contribute an additional 0.25 FTE
to the curator position. The 0.25 FTE would be used to help maintain teaching
collections. Teaching collections receive heavy use and frequently need to be
repaired, remounted or replaced. The resultant 0.75 FTE would not be used for
new teaching assignments, but for curatorial duties. This proposition is in agree-
ment with the resolution passed unanimously by the South Dakota Academy of
Science in April, 2006 (Gabel and Hutcheson, 2006).

With additional personnel support, curators will have time to write grant
proposals for extramural funding. The three herbarium curators have already
been in discussions concerning a proposal to the National Science Foundation
for production of a database of all plant specimens from eastern South Dakota.
These databases would be incorporated into a master dataset to produce a search-
able database through the National Biological Information Infrastructure and
the Global Biodiversity Information Facility (GBIF).

CONCLUSIONS

Nothing can or will replace the taxonomic information and training that
is provided by natural history collections. Without these collections a major
component of the history and natural history of South Dakota will be lost. The
benefits of this knowledge are important to the inhabitants of the state as is this
contribution to the understanding of global biodiversity. Development of natu-
ral history collections will undoubtedly produce new and unpredicted benefits.
Failure to support natural history collections is certain to eliminate current and
potential benefits. Those who sacrifice permanent values for short-term political
expediency must be held accountable for their actions (Raven, 2003).

ACKNOWLEDGEMENTS

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Ramsay and the staff of the W. H. Over Museum.

LITERATURE CITED


USE OF SWITCHGRASS SEED AS WILD BIRD FEED

Travis J. Runia and Daniel E. Hubbard
Department of Wildlife and Fisheries Sciences

Arvid Boe
Department of Plant Science

South Dakota State University
Brookings, SD 57007

ABSTRACT

To date, switchgrass (Panicum virgatum L.) seed is only used for planting new fields. However, the highly anticipated widespread planting of switchgrass as biomass for biofuel production prompted this investigation into whether switchgrass seed could be used as wild bird feed. We compared the consumption of switchgrass seed to proso millet (Panicum miliaceum) seed by birds from ground trays at 3 traditional bird feeding sites. We also removed the proso millet seed from the feeding sites after 21 days to determine if consumption of switchgrass seed would increase when offered independently. More (P ≤ 0.05) proso millet seed was consumed by birds at all sites when compared to switchgrass seed. When switchgrass seed was offered independently, consumption increased significantly at only 1 site. Reasons for poor use of the switchgrass seed could be low digestibility, its small size, or other reasons such as poor taste. Future studies should evaluate whether switchgrass seed would be preferred when offered in a mixture as this sometimes increases digestibility. Seeds of other or new cultivars of switchgrass also should be evaluated.

Keywords
Switchgrass, switchgrass seed, biofuel, bird feed

INTRODUCTION

Switchgrass, a native warm-season perennial dominant of the tall grass prairie, has been identified as a highly promising source of cellulosic biomass for biofuel production in the Northern Great Plains (e.g., Lee and Boe 2005). Recent research indicated that the most sustainable biomass production system for switchgrass in eastern South Dakota was a single annual harvest after physiological maturity during early autumn (Casler and Boe 2003). Using that harvest system, biomass producers also could harvest a mature seed crop when they harvest the biomass. To date, switchgrass seed is only used for planting new fields. However, the highly anticipated widespread planting of switchgrass for biomass
prompted this investigation to determine whether switchgrass seed could be used as a source of wild bird feed.

Seed selection in granivorous birds is not well understood and varies among studies. Pulliainen (1965), Moss (1968), and Gardarsson and Moss (1968) found some gallinaceous birds selected food based on nutritional quality while Willson (1971) and Robel et al. (1974) reported that birds will eat seeds that are most abundant. Several studies have found that handling time dictated preference for seeds by sparrows (Emberizidae spp.) and northern cardinals (Cardinalis cardinalis) (Willson 1971, Willson and Harmeson 1973, Keating et al. 1992). Handling time is determined by food characteristics (size, shape) and bird capabilities (bill size and shape) (Abbot et al. 1975, Sherry and McDade 1982). When seeds are equally abundant, granivorous birds may still not select them solely on nutritional quality or ease of handling (Shuman et al. 1990). Other factors such as seed color and flavor may also be important (Robel et al. 1997). Because of the complexity of seed selection by granivorous birds, field testing of seeds as potential bird feed is important.

The objective of our study was to determine if switchgrass seed would be readily consumed by birds at traditional bird feeding sites when offered with proso millet and independently. Proso millet was selected for comparison because it is a readily consumed component in many commercial wild bird feeds.

**STUDY AREA**

Our study was conducted at 3 sites within 10 km of the City of Brookings in east-central South Dakota. All 3 sites had bird feeders that were regularly filled and maintained. We used sites that had established feeding stations in order to ensure that birds would find our feeders quickly. Site 1 was located in a residential yard within the city of Brookings. The site was dominated by residential houses and roads. Large green ash (Fraxinus pennsylvanica) and black walnut (Juglans nigra) were quite prominent in this area. A tube style feeder with niger (Guizotia abyssinica) seed and a suet feeder were maintained here. Site 2 was 1 km north of Brookings and site 3 was 10 km east of Brookings. Both sites were private acreages with numerous buildings with open yards containing scattered trees of several species and were well protected by large shelterbelts dominated by green ash. The landscape was rolling to slightly rolling with corn (Zea mays) and soybean (Glycine max) fields dominant with wheat (Triticum aestivum), hay, pasture, wetlands, and Conservation Reserve Program fields also present. Site 2 had an open platform bird feeder that was regularly filled with black-oil sunflower (Helianthus annuus) seeds as well as tube feeders containing niger and sunflower. Site 3 had 2 existing tube feeders filled with niger seed in 1, and black-oil sunflower in the other. At Site 3 black-oil sunflower seeds also were scattered on the ground daily.
METHODS

Initially, each site was equipped with a pair of hanging tube-style bird feeders with 1 feeder containing proso millet seed and the paired feeder containing switchgrass seed. From 1-31 March 2006 both seeds were rarely eaten by birds, even when the different sized interchangeable feeding ports were changed. This method was abandoned and wooden trays (30 cm by 45 cm) were fabricated to accommodate ground feeding birds. Each site was equipped with a pair of wooden trays with a 2 cm by 4 cm wooden edge around all 4 sides. Trays were placed on the ground at established bird feeding areas at sites. From 1-7 April 2006, 1 tray was filled with switchgrass seed and the paired tray was filled with proso millet seed. During this period, the trays were maintained with an abundance of food to habituate the birds to the feeding trays. From 8-28 April 2006, 200 g of each seed were placed in trays. Trays were emptied and refilled with 200 g of seed daily. The remaining seed was weighed to the nearest 0.1 g and daily seed consumption was calculated. From 29 April-7 May 2006 only switchgrass seed was offered to birds to determine if consumption of switchgrass increased when it was offered independently. A Wilcoxon sign-rank test was used for analyzing consumption when both seeds were offered concurrently. The 9 days when switchgrass seed was offered independently was compared to the previous 9 days when the feeds were paired using a Kruskal-Wallis test. A significance level of $\alpha = 0.05$ was used for all analyses. JMP 5.1 (SAS Institute Inc., Cary, NC) was used for all analyses.

RESULTS

Birds removed more ($P \leq 0.05$) proso millet seed than switchgrass seed from the feeding trays when offered simultaneously (Table 1). Significantly more ($P = 0.025$) switchgrass seed was removed at site 2 when offered independently (Table 2). However, site 1 showed no difference ($P = 0.848$) and site 3 marginally significantly decreased ($P = 0.058$) in switchgrass seed consumption when the switchgrass seed was offered independently versus paired with proso millet seed.

Table 1. Mean daily seed mass ± SE removed from the feeding trays from 8-28 April 2006.

<table>
<thead>
<tr>
<th>Site</th>
<th>Switchgrass (g)</th>
<th>Proso millet (g)</th>
<th>P</th>
<th>Za</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.53 ± 2.03</td>
<td>109.80 ± 4.62</td>
<td>&lt; 0.001</td>
<td>115.5</td>
</tr>
<tr>
<td>2</td>
<td>19.39 ± 2.90</td>
<td>117.16 ± 8.67</td>
<td>&lt; 0.001</td>
<td>115.5</td>
</tr>
<tr>
<td>3</td>
<td>32.29 ± 8.38</td>
<td>168.94 ± 10.00</td>
<td>&lt; 0.001</td>
<td>126.5</td>
</tr>
</tbody>
</table>

* Wilcoxon sign-rank test.
DISCUSSION

Our results indicate switchgrass seed is less preferred by birds than proso millet seed although it was utilized to some degree. Even when the switchgrass was offered independently, only 1 site showed a significant increase in removal. Site 3 even showed a nearly significant decrease in switchgrass seed removal when offered independently.

We suspect nutritional quality or ease of handling caused the preference for the proso millet seed over the switchgrass seed because they were both equally available during the first part of the study. The larger size of the proso millet seed may enable birds to gather them more quickly and with less effort. Very small birds that may not be able to effectively forage on the proso millet seed may explain why the switchgrass was consumed in small amounts. Although no observational data were collected, we regularly observed small birds, such as chipping sparrows (Spizella passerine) and dark-eyed juncos (Junco hyemalis), utilizing the switchgrass seed and proso millet seed. Larger birds, such as mourning doves (Zenaida macroura), common grackles (Quiscalus quiscula), white-throated sparrows (Zonotrichia albicollis), and white-crowned sparrows (Zonotrichia leucophrys), were occasionally observed utilizing the switchgrass seed.

Although gross energy content of these 2 seeds is quite comparable, the metabolic energy efficiency of the proso millet seed is much higher. Madison and Robel (2001) found gross energy content of switchgrass and proso millet to be 4,506 and 4,417 cal/g respectively. These results were similar to those of Willson (1971), Shuman et al. (1988), and Saunders and Parrish (1987). Metabolic energy efficiency for proso millet seed ranges from 83.7% in mourning doves (Zenaida macroura) (Shuman et al. 1988) to 79.8% in northern bobwhites (Colinus virginianus) (Madison and Robel 2001). Saunders and Parish (1987) reported a metabolic energy efficiency of 61.7% for switchgrass seed in scaled quail (Callipepla squamata) and Heffron and Parrish (2005) found greater prairie-chickens (Tymanuchus cupido) only utilized 42.0% of the available energy in switchgrass seed. When northern bobwhites were fed only switchgrass seed or proso millet seed in a laboratory setting those fed switchgrass lost 7.0% of their body weight while body weights of those fed proso millet seed remained nearly unchanged (Madison and Robel 2001). These same birds ate less switchgrass seed per day than proso millet seed when they were offered in excess. In a similar study, scaled quail consumed less switchgrass seed when compared to 11 other seed species and also lost significant body weight (Saunders and Parrish 1987).

Table 2. Mean daily switchgrass seed mass ± SE removed from the feeding trays when offered independently or paired with proso millet.

<table>
<thead>
<tr>
<th>Site</th>
<th>Paired (g)</th>
<th>Independent (g)</th>
<th>P</th>
<th>$\chi^2$ Approximation, df</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.21 ± 3.08</td>
<td>21.31 ± 4.08</td>
<td>0.848</td>
<td>0.037, 1</td>
</tr>
<tr>
<td>2</td>
<td>10.13 ± 3.55</td>
<td>45.51 ± 10.99</td>
<td>0.025</td>
<td>5.000, 1</td>
</tr>
<tr>
<td>3</td>
<td>45.42 ± 17.77</td>
<td>12.49 ± 2.71</td>
<td>0.058</td>
<td>3.604, 1</td>
</tr>
</tbody>
</table>

*Kruskal-Wallis test.*
Due to its low preference and low digestibility by birds, switchgrass seed may not be effective bird feed. Future studies should explore whether certain species of birds show a preference for switchgrass seed. There may be some small-billed birds that prefer the small size of switchgrass seed because their bill size limits the size of seed they can consume. Yet, the low digestibility of this seed may still limit its benefits to birds even if it is readily consumed.

Future studies should also investigate the use of switchgrass seed in a mixture of other seeds. Multiple studies (Madison and Robel 2001, Saunders and Parrish 1987) have found substantial increases in metabolic energy efficiencies of seeds when birds consume them in a mixture rather than alone. Additionally, the low metabolic efficiency may be due to an energetic dilution effect of the higher proportion of seed coat to seed contents. Large genetic differences occur among populations of switchgrass for seed size (Boe 2007), and heritability for seed size is high in large-seeded populations of switchgrass (Boe 2003). Therefore, agronomic breeding for increased seed size also is of consideration.

ACKNOWLEDGEMENTS

We would like to thank Dr. K.C. Jensen and Dr. Jon A. Jenks for their reviews and suggestions on this manuscript. Their reviews greatly enhanced this paper.

LITERATURE CITED


EFFECTS OF A PROPRIETARY YEAST SUPPLEMENT DURING REARING OF TWO STRAINS OF JUVENILE RAINBOW TROUT AND JUVENILE LAKE TROUT

Michael E. Barnes and Brian Fletcher
South Dakota Department of Game, Fish and Parks
Spearfish, SD 57783

Dan J. Durben
Black Hills State University
Spearfish, SD 57799

ABSTRACT

Three experiments were undertaken to evaluate the effects of supplementation with yeast-based DV Aqua™ (Diamond V Mills, Cedar Rapids, IA) during the rearing of juvenile McConaughy strain rainbow trout *Oncorhynchus mykiss* (3 g initial weight), Shasta strain rainbow trout (2 g), and lake trout *Salvelinus namaycush* (7 g). Inclusion of 0.25% DVAqua into commercially prepared trout foods resulted in improved lake trout growth after 73 rearing days. Mortality was reduced by 50% in McConaughy strain rainbow trout after 37 rearing days, but no significant differences in growth were observed. The Shasta strain experiment lasted only 27 days, with no significant differences in growth or mortality noted between the fish receiving 0.25% DVAqua or a control diet. Dietary DVAqua is recommended during salmonid rearing, particularly for those species and strains which are more reluctant to consume commercially-produced aquaculture feeds.

Key Words

Yeast culture, DVAqua, rainbow trout, lake trout, *Salvelinus namaycush*, *Oncorhynchus mykiss*

INTRODUCTION

Dietary supplementation with a variety of yeasts and yeast-products during hatchery rearing has been evaluated with a number of different fish species. In salmonids, red yeasts, such as *Phaffia* spp., were first used to change muscle coloration (Johnson et al. 1977, 1980; Sanderson and Jolly 1994; Whyte and Sherry 2001; Storebakken et al. 2004). These yeasts also improved rainbow trout *Oncorhynchus mykiss* liver function, but had no effect on growth (Nakano et al. 1995, 1999; Whyte and Sherry 2001). In contrast, brewer’s (or baker’s) yeast *Saccharomyces cerevisiae* does not contain coloration-changing pigments, but
has been used as an alternative protein source to fish meal (Rumsey et al. 1990, 1991) and also has immunostimulant properties (Siwicki et al. 1994; Ortuño et al. 2002; Li and Gatlin 2004, 2005).

Barnes et al. (2006a, 2006b) noted significant improvements in salmonid survival and growth with the inclusion of DV Aqua™, a S. cerevisiae-based fermented yeast culture, in the diet during the initial feeding period. These trials lasted only through the first month or two of feeding, with the fish typically weighing much less than 1g at the end of the study period. We are unaware of any studies examining the use of brewer’s yeasts as a supplement in conjunction with commercially-prepared, fish-meal-based diets during the hatchery rearing of larger juvenile salmonids. Thus, the objective of this study was to determine the effect of a dried, fully fermented yeast culture supplement (DVAqua, Diamond V Mills, Cedar Rapids, IA) on the growth and survival of juvenile lake trout Salvelinus namaycush, and two strains of rainbow trout during short intervals of hatchery rearing.

METHODS

All experiments were conducted at McNenny State Fish Hatchery, Spearfish, South Dakota, USA. Well water at a constant temperature of 11°C (total hardness as CaCO₃, 360 mg L⁻¹; alkalinity as CaCO₃, 210 mg L⁻¹; pH, 7.6; total dissolved solids, 390 mg L⁻¹) was used throughout rearing in fiberglass circular tanks (1.8 m diameter, 0.8 m depth). The control diet was Silver Cup Trout Food (Nelson and Sons Inc., Murray, Utah), and the experimental diet was the Silver Cup food mixed with 0.25% DVAqua. Feeding rates were based on the hatchery constant method (1.1 feed conversion) and feed amounts were updated weekly (Buterbaugh and Willoughby 1967; Piper et al. 1982). Feed was uniformly dispensed from 07:00 to 19:00 in each tank using automatic EWOS 505 feeders (Norco-plast AB, Sweden) electronically programmed to release small amounts of feed for 2 min with 20-min intervals. All feed dispensed and fish deaths were recorded for each tank. Percent mortality was determined by dividing the number of fish that had died by the total number of fish in each tank. Individual fish lengths were measured to the nearest mm and weights recorded to the nearest g. Total tank weights to the nearest 0.05 kg were recorded at both the start and end of the experiment.

Experiment 1. McConaughy strain rainbow trout

On June 7, 2004, 6.8 kg of McConaughy strain rainbow trout (approximately 2,300 fish based on the average weight per individual fish) were placed into each of eight circular tanks. These fish had been on feed for approximately three months and had attained a length of 65 mm. Four tanks received 1.5-mm Silver Cup Extruded Floating Trout Food, while another four tanks received the Silver Cup diet pre-mixed with DVAqua. Feeding rates were based on a hatchery constant of 6.6. Total lengths and weights were recorded from 30 fish obtained from the common pool of fish prior to placement into the circular tanks at the
start of the experiment. Twenty fish from each tank \( (N = 80/\text{treatment}; 20 \text{ fish from each of four tanks}) \) were weighed and measured at the end of the experiment on July 14, 2004.

**Experiment 2. Shasta strain rainbow trout**

On April 28, 2004, 17.0 kg of domesticated Shasta strain rainbow trout (approximately 10,800 fish based on the average weight per individual fish) were placed into each of eight tanks. These fish had been on feed for approximately two months and had attained a size of 54 mm. Four tanks received #2 Silver Cup trout granules, while another four tanks received the Silver Cup diet post-production mixed with DVAqua. Feeding rates were based on a hatchery constant of 6.6. Total lengths and weights were recorded from 80 fish obtained from the common pool of fish prior to placement into the circular tanks at the start of the experiment. Ten fish from each tank \( (N = 40/\text{treatment}; 10 \text{ fish from each of four tanks}) \) were weighed and measured at the end of the experiment on May 25, 2004.

**Experiment 3. Lake Trout**

On May 19, 2004, 9.1 kg of lake trout (approximately 1,240 fish based on the average weight per individual fish) were placed into each of eight tanks. These fish had been on feed for approximately four months and had reached a length of 100 mm. Four tanks received 1.5-mm Silver Cup Extruded Floating Trout Food, while another four tanks received the Silver Cup diet pre-mixed with DVAqua. Feeding rates were based on a hatchery constant of 7.26. Total lengths and weights were recorded from 80 fish obtained from the common pool of fish prior to placement into the circular tanks at the start of the experiment. Twenty fish from each tank \( (N = 80/\text{treatment}; 20 \text{ fish from each of four tanks}) \) were weighed and measured at the end of the experiment on July 13, 2004.

**Statistical Analysis**

Data were analyzed by Student’s t-tests with significance predetermined at \( P < 0.05 \). All mortality percentage data were arcsine transformed prior to analysis to stabilize the variances (Ott 1984).

**RESULTS**

Only 0.22% of McConaughy rainbow trout died in the tanks receiving DVAqua, which was significantly less than the 0.44% mortality observed in the control tanks (Table 1). Other total tank data were nearly identical between the treatments. Ending weights, gain, and feed conversion were not significantly different. Individual fish lengths and weights were not significantly different between the fish receiving either the control diet or DVAqua supplementation (Table 2). Condition factor was significantly greater in the control fish however.
No significant differences were observed between the tanks fed 0.25% DVAqua or the control diet during the Shasta strain rainbow trout experiment (Table 3). Total tank ending weights, gain, feed conversion, or mortality were not significantly different. There was also no significant difference in individual fish length, weight, or condition factor between the treatments (Table 4).

Although mean total tank ending weight, gain, and feed conversion were all slightly improved in the lake trout tanks fed 0.25% DVAqua, they were not significantly different from the control tanks (Table 5). Percent mortality was very similar between the two diets tested. When individual fish fed diets either with or without the Diamond V yeast product were weighed and measured, there were significant differences (Table 6). On average, mean ending weights were nearly 20% greater in the fish fed 0.25% DVAqua compared to the non-yeast culture.
fed fish. Although mean total lengths were over 3 mm longer in the fish eating yeast-containing food, this was not statistically different from the fish fed diets with no yeast product. Condition factors were also significantly improved in the fish fed yeast diets.

**DISCUSSION**

The positive influence on survival observed with the McConaughy strain rainbow trout may be an extension of the dramatic decrease in mortality reported by Barnes et al. (2006a) for the same strain during initial feeding. The McConaughy strain is relatively difficult-to-grow, yet exhibits exceptional survival and growth in waters containing predators and competitors (R. Hanten, personal communication). In contrast, the Shasta strain is probably the most domesticated of all rainbow trout strains, and was one of the first strains developed for

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**Table 3.** Mean (SE) weight gain, feed conversion, and mortality rates for tanks of fingerling Shasta strain rainbow trout fed diets with or without DVAqua.

<table>
<thead>
<tr>
<th>DVAqua Supplementation</th>
<th>None</th>
<th>0.25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N )</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Start weight (kg)</td>
<td>17.0</td>
<td>17.0</td>
</tr>
<tr>
<td>End weight (kg)</td>
<td>35.7 ± 0.1</td>
<td>36.2 ± 0.8</td>
</tr>
<tr>
<td>Gain (kg)</td>
<td>18.7 ± 0.1</td>
<td>19.2 ± 0.1</td>
</tr>
<tr>
<td>Food fed (kg)</td>
<td>15.6</td>
<td>15.6</td>
</tr>
<tr>
<td>Conversion</td>
<td>0.84 ± 0.01</td>
<td>0.81 ± 0.01</td>
</tr>
<tr>
<td>% mortality</td>
<td>0.25 ± 0.01</td>
<td>0.19 ± 0.01</td>
</tr>
</tbody>
</table>

**Table 4.** Mean (SE) weights, lengths, and condition factors (K)\(^a\) of fingerling Shasta strain rainbow trout fed diets with or without DVAqua.

<table>
<thead>
<tr>
<th>DVAqua Supplementation</th>
<th>None</th>
<th>0.25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N ) start</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>( N ) end</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Start weight (g)</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>End weight (g)</td>
<td>3.0 ± 0.2</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>Start length (mm)</td>
<td>54.1 ± 0.5</td>
<td>54.1 ± 0.5</td>
</tr>
<tr>
<td>End length (mm)</td>
<td>66.3 ± 1.0</td>
<td>67.4 ± 0.7</td>
</tr>
<tr>
<td>Start K</td>
<td>0.93 ± 0.01</td>
<td>0.93 ± 0.01</td>
</tr>
<tr>
<td>End K</td>
<td>1.00 ± 0.01</td>
<td>1.03 ± 0.01</td>
</tr>
</tbody>
</table>

\(^a\) Condition factor (K) = 10\(^5\) x (weight)/(length\(^3\))
The improvements in growth observed in the lake trout receiving dietary DVAqua follows the pattern observed by Barnes et al. (2006a, b) and in this study with the two strains of rainbow trout, with DVAqua effects more apparent in difficult-to-rear fish. DVAqua may be acting as a feed stimulant or attractant, enhancing the palatability of the commercially-prepared diet for fish species and strains that are reluctant to eat non-natural foods. Palatability of fish food is influenced by amino acid levels (Adron and Mackie 1978; Johnsen and Adams 1986; Harada 1989; Heinsbroek and Kreuger 1992; Papatryphon and Soares 2000), which yeasts typically have in abundance (Shcherbina et al. 1987; Ap pelbaum 1979; Cheng et al. 2004). DVAqua, as a fully fermented yeast culture, contains *S. cerevisiae* and a range of unidentified metabolites that could serve a

### Table 5. Mean (SE) weight gain, feed fed, feed conversion, and mortality rates for tanks of fingerling lake trout fed diets with or without DVAqua. Means with different letters are significantly different (P < 0.05).

<table>
<thead>
<tr>
<th>DVAqua Supplementation</th>
<th>None</th>
<th>0.25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Start weight (kg)</td>
<td>9.1</td>
<td>9.1</td>
</tr>
<tr>
<td>End weight (kg)</td>
<td>24.9 ± 0.1</td>
<td>25.3 ± 0.3</td>
</tr>
<tr>
<td>Gain (kg)</td>
<td>15.8 ± 0.1</td>
<td>16.2 ± 0.3</td>
</tr>
<tr>
<td>Feed fed (kg)</td>
<td>12.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Conversion</td>
<td>0.76 ± 0.01</td>
<td>0.74 ± 0.01</td>
</tr>
<tr>
<td>% mortality</td>
<td>0.30 ± 0.11</td>
<td>0.36 ± 0.05</td>
</tr>
</tbody>
</table>

### Table 6. Mean (SE) weights, lengths, and condition factors (K) of fingerling lake trout fed diets with or without DVAqua. Means with different letters are significantly different (P < 0.05).

<table>
<thead>
<tr>
<th>DVAqua Supplementation</th>
<th>None</th>
<th>0.25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Start weight (g)</td>
<td>7.6 ± 0.3</td>
<td>7.6 ± 0.3</td>
</tr>
<tr>
<td>End weight (g)</td>
<td>19.7 ± 0.6 z</td>
<td>23.5 ± 0.8 y</td>
</tr>
<tr>
<td>Start length (mm)</td>
<td>100.4 ± 1.1</td>
<td>100.4 ± 1.1</td>
</tr>
<tr>
<td>End length (mm)</td>
<td>134.8 ± 1.6</td>
<td>138.0 ± 1.3</td>
</tr>
<tr>
<td>Start K</td>
<td>0.73 ± 0.01</td>
<td>0.73 ± 0.01</td>
</tr>
<tr>
<td>End K</td>
<td>0.78 ± 0.01 y</td>
<td>0.87 ± 0.01 y</td>
</tr>
</tbody>
</table>

*a Condition factor (K) = 10^5 x (weight)/(length^3)*
similar function. If DVAqua is indeed palatability-enhancing, its relatively low cost would be atypical compared to other feed stimulant ingredients (Barrows and Hardy 2001).

DVAqua may also have increased lake trout growth in other ways. The *S. cerevisiae* in DVAqua is a protein source by conventional definition (Cheng et al. 2004) and in non-salmonids, fish growth has improved with dietary supplementation of *S. cerevisiae* and other yeast species (Noh et al. 1994; Oliva-Teles and Goncalves 2001; Lara-Flores et al. 2002; Li and Gatlin 2003, 2004). In addition to direct nutritional benefits, yeast may also improve fish nutrition indirectly by adhering to fish intestinal mucus and producing polyamines (Vázquez-Juárez et al. 1993; Tovar et al. 2002; Tovar-Ramírez et al. 2004). Lastly, it is also possible that the increased growth in lake trout associated with dietary DVAqua was due to immunomodulatory effects. Yeast in general has been shown to improve immunological function in fish (Siwicki et al. 1994; Nakano et al. 1995, 1999; Ortuño et al. 2002; Li and Gatlin 2004, 2005) and the fully fermented yeast culture, DVAqua in particular improved the disease resistance of Pacific white shrimp (Burgents et al. 2004).

The results from our three experiments should be interpreted with regard to the size of fish used, the relatively short duration of each experiment, and the relatively small sample sizes resulting in low statistical power (Curtis et al. 1991). Longer experiments encompassing a greater range of fish sizes with additional replication may produce results different than we observed.

The lake trout results, in which the non-significantly-different total tank data appears not to support the significantly-different individual fish measurements, can likely be explained by the relatively small number of tanks. Larger sample sizes with the individual fish data improve the ability to ascertain statistically significant differences. It is also possible that the yeast-containing diet produced benefits for a sub-population of the entire tank, perhaps skewing the weight and condition factor results. Even if this was the case, it would still indicate that there were some benefits to yeast culture supplementation with this salmonid species. It is also possible that the study duration was too short for the small, but significant differences in individual fish size to become statistically observable in the total tank weights.

On the basis of our experiments, where DVAqua improved survival in McConaughy strain rainbow trout, improved growth in lake trout, and did no apparent harm to Shasta strain rainbow trout, we recommend the inclusion of DVAqua in salmonid diets. Additional experiments will likely ascertain the best inclusion rates and also illuminate the long-term effects of DVAqua supplementation.

ACKNOWLEDGEMENTS

We thank Greg Simpson and Ilkyu Yoon for their review of this manuscript. We also thank Rachel Sanders, Will Sayler, Rick Cordes, and Eric Krebs for their culture assistance, the many Black Hills State University students who
participated in this study, and the reference librarians at the South Dakota State Library. This study was supported by Diamond V Mills, Cedar Rapids, IA.

REFERENCES


FUTURE WATER SUPPLIES FOR RAPID CITY

P.H. Rahn
Department of Geology and Geological Engineering
South Dakota School of Mines and Technology
Rapid City, SD 57701

ABSTRACT

Municipal water use for the City of Rapid City, South Dakota, averaged 22.92 cubic feet per second (cfs) from 2000 to 2005. This water was derived from eight wells largely withdrawing from the Madison Limestone (9.95 cfs), two galleries in alluvium along Rapid Creek (2.75 cfs), Jackson Spring (9.23 cfs), and Rapid Creek (0.99 cfs). In the next 30 years municipal water demand may double, from approximately 23 cfs in 2005 to 46 cfs in 2035.

In 2006 Rapid City acquired surface water rights to Rapid Creek through the U. S. Bureau of Reclamation storage agreement for 78 cfs from Pactola and Deerfield Reservoirs. However, during years of normal precipitation the discharge of Rapid Creek above Canyon Lake is only approximately 42 cfs. Nevertheless, because of this water rights acquisition, Rapid City has an adequate water supply for the next 30 years, not considering droughts.

There are plans to drill more municipal wells to the Madison Limestone in the western part of Rapid City. But the long-term availability of ground water is affected by: (1) withdrawals by industrial users, (2) the threat of contamination because of short travel times from stream recharge sites to municipal wells, and (3) the increasing nitrate concentration in municipal wells, presumably from upgradient domestic wastewater systems. In the short term, wells in the Madison and Minnelusa aquifers could probably supply 26 cfs for municipal supply, but in the long term these withdrawals would compromise local springs, e.g. the Cleghorn/Jackson Spring complex.

The long-term water supply should combine surface water (Rapid Creek) and ground water. Primary reliance should be made on surface water. Secondary reliance should be ground water to be utilized during drought when Rapid Creek has low discharge.

Key words
Rapid City, water supply, Madison Limestone

INTRODUCTION

This paper is a result of contributions I made to the Rapid City Utility System Master Plan by Burns and McDonnell (2006a, 2006b). This paper reflects my own findings and recommendations and does not necessarily represent those
of Burns and McDonnell or the City of Rapid City. I thank Arden Davis and Stacey Titus for their editorial comments.

The municipal water supply of Rapid City consists of eight wells, two infiltration galleries and one spring (Figure 1). In the summer, when demand is great, water is also obtained directly from Rapid Creek; the intake is at the water

Figure 1. Map of the western part of Rapid City showing locations of water supply sources. Modified from Greene (1999) and Anderson et al. (1999). The Madison Limestone is highlighted; its potentiometric surface (feet above sea level) and general ground water flow direction are shown. MB = Meadowbrook Gallery, GS = Girl Scout Gallery, and JS = Jackson Spring.
treatment plant below the Girl Scout Gallery. Monthly discharge data from 2000 to 2005 for the municipal water supply is contained in supporting documents provided by Burns and McDonnell (2006a; 2006b). The water utilized from these sources varies every month, but for the purpose of this paper discharge is summarized as the average use during these six years. The municipal water use totals 22.9108 cfs. This discharge is derived from four general sources:

(1) Jackson Spring produced 9.2255 cfs.
(2) The eight wells produced 9.9510 cfs.
(3) The two galleries produced 2.7487 cfs.
(4) An average of 0.9856 cfs was taken directly out of Rapid Creek.

The Figure 2 shows these four water sources plotted at their approximate locations in Rapid City.

HYDROGEOLOGY

The movement of ground water in the bedrock aquifers on the eastern side of the Black Hills is generally easterly. Figure 3 shows the potentiometric surface of the Madison Limestone in this area. It is approximately 3,400 feet above sea

Figure 2. Map of Rapid City and the surrounding area showing water-supply facilities and USGS gaging stations (modified from Anderson et al., 1999). The four sources of municipal supply are shown with their approximate discharge from year 2000 through 2005. The total water use is 22.91 cfs.
level near well RC-9, for example. The ground-water flow direction in this aquifer is generally perpendicular to the potentiometric lines.

An important hydrogeologic factor in this area is the ground-water recharge from three streams that lose water into sinkholes near Rapid City. Figure 3 shows the Madison outcrop area where most of the recharge occurs. The sinkhole loss zones, where the three streams lose water, vary seasonally. Most of the time water from Boxelder Creek sinks dramatically just above the Nemo Road bridge in Section 16, T 2 N, R 6 E. During higher discharge the water sometimes flows downstream four miles before drying up. Rapid Creek seems to lose a constant 10 cfs in the Dark Canyon reach (Long and Putnam, 2002). Rapid Creek never dries up completely in this reach. Spring Creek loses most of its water below the Stratobowl (Section 1, T 1 S, R 6 E), but during high discharge water continues approximately three miles downvalley before disappearing. Figure 3 also shows the general ground water flow direction from the recharge areas of Boxelder Creek, Rapid Creek, and Spring Creek as deduced from dye tests and isotope studies by the U.S. Geological Survey.

**GROUND WATER AND WELLS**

Rahn and Gries (1973) described the hydrogeology of the Black Hills and made initial discharge measurements of Cleghorn Spring and City Spring in
Rapid City. The locations of these two springs are shown on Figure 2. Dye was used to trace the movement of water from sinkholes at Boxelder Creek into the Rapid Creek drainage. Greene (1999) confirmed that Boxelder Creek water appeared in City Spring and RC-6 in 30 days; it appeared in RC-10 in 41 days. The significance of these dye tests is that they confirm a connection between Boxelder Creek and the Rapid City water supply. Water moves quickly through the Paleozoic carbonate aquifer from the Boxelder Creek drainage basin to the Rapid Creek drainage basin. Boxelder Creek originates in Meade and Lawrence Counties, and Rapid City’s water supply is vulnerable to contamination that might occur in the Boxelder Creek drainage.

In the last decade the hydrology of the Black Hills has been intensely studied by the U.S. Geological Survey. Carter et al. (2002), Driscoll et al. (2002), and Carter et al. (2003) summarized some of the USGS research. Related USGS reports are listed in the References Cited section of these three reports and in the References given in this paper.

Long and Putnam (2002) described the hydrogeology of the western part of Rapid City, and included an analysis of the ground-water flow patterns in the bedrock aquifers utilized in Rapid City’s water supply. Figure 3 shows the interpreted flow paths from water recharged by Boxelder, Rapid, and Spring Creeks. Long and Putnam (2002) used dye tests as well as stable isotopes ($^{18}$O and $^2$H) for Boxelder Creek to indicate source areas and flow paths, whereas hydrogeology and isotope indicators were used for Rapid and Spring Creeks.

Miller (2005) studied the hydrogeology near Rapid City, including the effect of Boxelder, Rapid, and Spring Creeks, on recharging the Madison aquifer and thereby contributing to Rapid City’s Madison wells. Geologic maps at 1:24,000 scale show the location of relevant hydrogeologic features including domestic wastewater systems.

Until 1999, nine wells were used for the municipal supply. These include three older wells in the Minnelusa Formation (RC-1, RC-3, and RC-4), and six newer wells (RC-5, RC-6, RC-8, RC-9, RC-10, and RC-11) in the Madison Limestone. Since 1999 well RC-3 has not been used. Another well, RC-12, drilled to the Madison Limestone approximately 1 mile southeast of RC-11, has not yet been connected to the city water system. Well RC-7 was abandoned after it was drilled due to low yield.

Rahn (2006) showed municipal wells dates of construction, total depth, and approximate pumping rate from 1986 to 1997; the total well withdrawal during these years was 4.75 cfs. From 2000 to 2005 the total well withdrawal was 9.9510 cfs. Pumping test data are shown by Greene (1993), Greene and Lamb (1994), Anderson et al. (1999) and Burns and McDonnell (2006a). The sum of the discharges of the eight operational wells is 10,315 gpm, equivalent to 23.0 cfs.

**MEADOWBROOK AND GIRL SCOUT GALLERIES AND JACKSON SPRING**

The Meadowbrook and Girl Scout galleries consist of collection pipes laid horizontally into the alluvium along Rapid Creek. Anderson et al. (1999) found
that Meadowbrook Gallery produced 2.9 cfs during water year 1988-89 and Girl Scout Gallery produced 1.2 cfs. From 2000 to 2005 Girl Scout produced 1.04 cfs and Meadowbrook produced 1.71 cfs. Much of this water is obtained by induced infiltration from Rapid Creek, although some is derived from ground water flowing downvalley through the alluvium. Anderson et al. (1999) reported that “hydrochemical data confirm the presence of creek water resulting from induced infiltration at both galleries…” but the “effects on Rapid Creek were difficult to quantify because of relatively small pumping rates, large alluvial influences, and large streamflow variability…” The City of Rapid City utilizes surface water right permits (from Pactola Reservoir) for all water pumped from the two galleries (John Wagner, pers. comm., June 6, 2006).

The hydrogeology of Jackson Spring was described by Anderson et al. (1999). The water rises and emanates through a “breccia pipe” (collapse structure) originating in the Madison Limestone and/or Minnelusa Formation. Cleghorn Spring is only 400 ft away, and the two springs are essentially the same water (Long and Putnam, 2002). They are referred to as the “Jackson-Cleghorn complex” (Anderson et al., 1999). The water from Cleghorn Spring is collected and runs by gravity into the Cleghorn Spring Fish Hatchery that is operated by the S.D. Department of Game, Fish and Parks. In 2005 the City acquired 0.89 cfs water right from the Cleghorn Water Users; this water formerly was diverted from Cleghorn Spring for private use. The total discharge from the Cleghorn/Jackson spring complex was 21.6 cfs during water years 1988-89 (Anderson et al., 1999).

Jackson Spring supplied 6.0 cfs to Rapid City’s municipal water before 2000 (Anderson et al., 1999), and from 2000 to 2005 it supplied an average 9.2 cfs. Jackson Spring is along the bank of Rapid Creek, and the municipal pump installed into the spring induces infiltration from Rapid Creek, estimated at 10% of the production. When Jackson Spring was pumped heavily, drawdown was measured to be up 5 ft lower than the stage of Rapid Creek. The “operation of the gallery was believed to affect the flow of water available to the hatchery” (Anderson et al., 1999). The USGS research was not able to demonstrate that any water from Rapid Creek made its way into the hatchery water.

**WELL AND AQUIFER CHARACTERISTICS**

Rapid City’s six new operational Madison wells are in the Madison Limestone, a Mississippian-aged aquifer. The formation is up to 500 ft thick. The upper 100 to 200 ft, called the Madison aquifer by the USGS, has high permeability because of solution openings. According to Long and Putnam (2002), 25% of the wells completed to the Madison aquifer in the Black Hills are capable of producing 200 to 2,500 gpm. Rapid City withdrew an average of 3.8 cfs (2.5 mgd) from the Madison aquifer and 1.0 cfs (0.68 mgd) from the Minnelusa aquifer during the water years 1988 to 1997 (Long and Putnam, 2002). This was an estimated 48% of the water used from these aquifers in the Rapid City area. The other users include the (formerly State-owned) Dakotah cement plant, local homeowner groups, and industrial users including two large gravel quarries.
(Miller, 2005) and a Coca-Cola bottling plant. From 1988 to 1997, 3.44 cfs were withdrawn from the municipal Madison wells during the drier months, and 3.83 cfs during the wetter months. The most productive well, RC-9, averaged 2.579 cfs from 2000 through 2005.

The USGS conducted pumping tests on some of the new Madison wells. Long and Putnam (2002) provided detailed pumping test analyses for several wells. Greene and Rahn (1999) described a pumping test using wells RC-5 and RC-6. They found a principal transmissivity anisotropy of 1,500 m²/d in an N 42ºE direction, and a minor transmissivity of 120 m²/d at N 48ºW. The principal transmissivity agrees with major cave passageway directions in local caves. Anisotropic hydrogeological conditions surrounding the other municipal wells presumably exist but have not been studied.

WATER QUALITY

Williamson and Carter (2001) documented the water quality in the Black Hills. Isotopic analyses of surface and ground waters were given by Naus et al. (2001). Burns and McDonnell (2006b) showed detailed water quality data for Rapid City municipal sources. In general the municipal water for Rapid City has excellent quality. Rapid City has better quality water than most municipalities in South Dakota.

Total dissolved solids (TDS) are a measure of the dissolved constituents in water. In general, highly mineralized water is not desired for drinking water; the US EPA's secondary maximum contaminant level for TDS concentration is 500 mg/L (Williamson and Hayes, 2000). Water from the two galleries is slightly more mineralized than the water obtained from the wells or directly from Rapid Creek (Miller, 2005). The water from the Girl Scout and the Meadowbrook galleries shows total dissolved solids ranging from 392 to 484 mg/L. The dissolved minerals are largely calcium, magnesium, and bicarbonate. Sulfate ranges from 85 to 106 mg/L. The concentrations of all water quality parameters are less than drinking water standards. Complete water chemistry analyses including radiological data are collected less frequently.

Despite the relatively good quality of the municipal water, there is concern because the Madison aquifer is very permeable and hence contaminants could travel swiftly through this aquifer. For example, in November, 1993, the nearby Copper Oaks subdivision well (SW ¼ Section 5, T 1 S, R 6 E) in the Madison Limestone near Spring Creek was found to have Giardia bacteria (Miller, 2005).

Nitrate data are collected annually for all the Rapid City water sources. Nitrate is important because it is an indicator of sewage, fertilizers, or barnyard contamination (Hitt and Nolan, 2005). Coker (1981), Musa (1984), Meyer (1987), and Sawyer (in prep.) documented contamination of Black Hills ground water from sewage. Rahn (2006) showed rising nitrate concentrations in the six Madison wells. The rising nitrate is of concern because it most likely comes from on-site wastewater systems. Pharmaceutical compounds are associated with sew-
age (Stone and Heglund, 2005) and there is concern that they might appear in the Rapid City water supply.

Sawyer and Cowman (2000) described the “Source Water Assessment and Protection” program in the Black Hills. A report by the South Dakota Department of Environment and Natural Resources (2003) includes a contaminant inventory and maps of potential contamination sites. The source area for Rapid City’s water supply includes drainages of Boxelder, Rapid and Spring Creeks because the three streams all contribute water to Rapid City’s supply. Contamination that occurs on these three watersheds would ultimately affect Rapid City’s municipal supply.

Hortness and Driscoll (1998), Carter et al. (2001a) and Putnam and Long (2005) documented that, in the recharge (“sinkhole”) zones of these streams, water is lost to the Madison and Minnelusa aquifers. The following rates of recharge were determined by Carter et al. (2001b, Table 10 and 17):

Boxelder Creek (years 1967-1998): 14.24 cfs,
Rapid Creek (years 1992-1998): 10.00 cfs,
Spring Creek (years 1992-1998): 15.02 cfs.

Long and Putnam (2002, Table 12), showed average recharge as:

Boxelder Creek: 14.9 cfs,
Rapid Creek: 10.0 cfs,
Spring Creek: 10.7 cfs.

For the purpose of this paper, recharge rates from these three streams can be rounded off as follows:

Boxelder Creek: 14 cfs,
Rapid Creek = 10 cfs,
Spring Creek = 15 cfs.

The sum is 39 cfs.

Sawyer and Lindquist (2003) identified more than 9,000 on-site wastewater treatment systems within the “source water area” for Rapid City. Many are near the three creeks above the recharge zones. On-site wastewater systems within a few miles west of Rapid City pose a threat to Rapid City’s new Madison wells. Ghannam and Rahn (1993), Carter et al. (2002) and Carter and Driscoll (2006) estimated precipitation recharge to the Paleozoic carbonate belt near Rapid City to be approximately 2 inches annually. Infiltrating domestic wastewater would be added to this natural recharge. Because of concern about rising nitrate concentrations, in 2006 the Rapid City Council enacted “On-site wastewater Disposal Ordinance No. 4083” to regulate on-site septic wastewater systems within the city limits and extending one mile beyond the perimeter of the city limits.

Within the three drainage basins that are the source area for Rapid City’s water supply, there are water quality concerns other than domestic wastewater.
This includes the Hill City sewage lagoons, gas stations, agricultural practices, and the proximity of highways to Boxelder, Rapid and Spring Creeks.

FUTURE WATER NEEDS

The long-term water requirements for Rapid City have been studied by the Rapid City Engineering Department (Rapid City Area Metropolitan Planning Organization, 1999; Miller, 2005). It is beyond the scope of this paper to include detailed forecasts of the water needs. Nevertheless, municipal water use rose as follows:

3.1 cfs in 1920,  
10.52 cfs in 1962,  
22.9 cfs in 2000-2005.

These data show that water use doubles about every 30 years. By extrapolation, the water needs by year 2035 would be about 46 cfs.

The future water supplies available for Rapid City involve an analysis of ground and surface waters.

The water rights for ground water from the Madison wells totals 8.01 cfs. [See “Water Rights Review and Updates” by Dan Bjerke, Appendix H in Burns and McDonnell (2006a).]

The term “safe yield” is used to convey the concept that there is a finite amount of water that can be withdrawn from an aquifer. In many of the more arid places in the United States ground water is withdrawn from an aquifer at a rate exceeding its recharge rate. Because of this, water is essentially being “mined”, and eventually will become depleted. The High Plains aquifer in Texas and Oklahoma and alluvial aquifers in southern Arizona have been exploited in this way. The Paleozoic carbonate aquifer underlies much of western South Dakota and has great quantities of stored water because of its thickness and areal extent. Carter et al. (2003) and Driscoll et al. (2002) showed 62.7 million acre-ft of ground water stored in the Madison Limestone and 70.9 million acre-ft stored in the Minnelusa Formation extending roughly 20 miles from the Black Hills. For this paper, in order to evaluate the long-term availability of ground water in the Rapid City area it is assumed that mining ground water, resulting in widespread drawdown of water levels, is not desirable. Therefore the volume of water stored in the Paleozoic carbonate aquifer is essentially irrelevant to the evaluation of the safe yield of this aquifer. Rather, recourse should be made to its recharge rate, and the safe yield determined by evaluation of the recharge rate in light of possible impacts to springs caused by ground-water withdrawals.

Ground-water recharge to the Paleozoic carbonate aquifer in the western Rapid City area is primarily from three streams. Stream discharge has been studied by Rahn and Gries (1973), Miller and Driscoll (1998), Hortness and Driscoll (1998), and others. Figure 3 shows that water recharged by Spring, Rapid and Boxelder Creeks makes its way into the general cone of depression caused by the Cleghorn/Jackson Spring complex and by Rapid City’s Madison wells. One
method of determining the ultimate (long-term) availability of ground water is to assume that the amount of water withdrawn from an aquifer is limited by its recharge rate. As discussed above, the sum of the recharge by these three streams is 39 cfs. Because Rapid City’s withdrawals represent about 50% of the withdrawals in this area, approximately 50% of the recharge, or 20 cfs, would be the share of this recharge that could reasonably be expected by the municipal wells. This is a high estimate of the availability of ground water for Rapid City municipal supplies because this amount of ground-water withdrawal would affect springs in this area.

Another way of estimating the long-term availability of ground water from the Paleozoic carbonate aquifer is to consider local spring discharge. Several springs in the Rapid City area are the natural overflow points of an aquifer system that is essentially “full”. The springs are recharged by disappearing streams and by precipitation. [Some of the ground water in these aquifers moves downgradient away from the Black Hills, estimated at 1.3 cfs in the Rapid City area (Carter et al., 2001a).] Water in excess of the discharge downgradient through the aquifer is discharged as springs. Cleghorn/Jackson Spring complex, City Spring, and numerous small springs near the cement plant are the only springs of this type within about 30 miles of Rapid City; Carter et al. (2001a, Table 5) showed that they total 25.6 cfs. Ground-water withdrawal at this rate in the Rapid City area represents a long-term sustained availability of water from these aquifers. Complete utilization of this discharge, however, ultimately would jeopardize the Cleghorn Fish Hatchery and other users.

According to Carter et al. (2002): “Artesian springs act as a ‘relief valve’ for the Madison and Minnelusa aquifers. Large-scale development of these aquifers could diminish artesian springflow, impacting surface-water resources, before large-scale declines in ground-water levels would occur.” A relevant example was the large 1970s pumping project proposed by Energy Transportation Systems, Inc. (ETSI) near Edgemont, SD. The USGS found that large ground-water withdrawals from the Madison Limestone would severely diminish the discharge of Cascade Spring (Konikow, 1976). A future Rapid City study, using ground-water modeling, would help evaluate the long-term availability of ground water.

City Spring, once the sole source of Rapid City’s water, provides a local example of pumping a well near a spring. RC-6, drilled in 1991, is only 100 ft away from City Spring, and pumping from RC-6 is most likely the reason why City Spring has been dry since 2004. The water level in a nearby USGS Madison well at City Quarry dropped from ground level to 70 ft below the land surface from 1999 to 2006. This drop could be caused partially by drought but is more likely because of pumping RC-6. [Note: In the late 1980s City Spring also reportedly dried up; therefore the reason for its recent demise is not certain.]

Figures 4 and 5 illustrate the concept that ground water withdrawn from the new Madison wells in the western part of Rapid City will most likely diminish the major spring in this region, the “Cleghorn/Jackson Spring complex”. Presently approximately 25 cfs emanates from this spring complex and several smaller nearby springs (Figure 4). Using this simplified model, in the future if an additional 23 cfs is withdrawn from nearby Madison wells, the discharge of the Cleghorn/Jackson Spring complex would be reduced to 2 cfs (Figure 5). A
Figure 4. Schematic geologic cross section showing hydrogeology and present (2005) ground-water use from the Madison Limestone in the western Rapid City area.

Figure 5. Schematic geologic cross section showing predicted future (2035) ground-water conditions if an additional 23 cfs is withdrawn from the Madison Limestone in the western Rapid City area.
detailed study is needed to thoroughly evaluate this scenario and to determine how long it will take for this impact to occur.

There are advocates for increased ground water withdrawals, but after the recent acquisition of surface-water rights, it seems logical that increasing reliance will be made on Rapid Creek for municipal water supply. The average discharge of Rapid Creek above Canyon Lake (just above Jackson Spring) is 41.65 cfs and at Rapid City is 57.57 cfs (Miller and Driscoll, 1998). But not all the Rapid Creek discharge would be available for municipal supply because this discharge includes flood events whose water could not be utilized. The annual allotment of 49,600 acre-ft from Pactola Reservoir and 7,000 acre-ft from Deerfield Reservoir became available to Rapid City by the 2006 contract with the U.S. Bureau of Reclamation (Burns and McDonnell, 2006b). Therefore, 56,600 acre-ft would theoretically be available annually (equivalent to 78.13 cfs). This is an unrealistically high estimate of the surface water available from Rapid Creek because it exceeds the average discharge. The USBR compact that gives 78 cfs from Pactola and Deerfield Reservoirs is for water that would be available provided there was sufficient precipitation that year to allow the USBR to disperse the water. Droughts are not factored into this arrangement; there is no guarantee of the water. Also, high-use days in the summer would have to be factored into the releases from Pactola.

Environmental factors have to be considered if increased reliance is made on Rapid Creek. For example, complete utilization of Rapid Creek would mean Rapid Creek would essentially become an ephemeral channel from the water intake plant all the way to the sewage treatment plant. The water quality of Rapid Creek could become degraded by street runoff (including lead compounds) above the water intake plant.

**SUMMARY**

The present use of Rapid City municipal water is approximately 23 cfs. In the next 30 years it could double to 46 cfs. Future water supply could be met by surface water and supplemented by ground water. If surface water is utilized from Rapid Creek, 77 cfs (estimate using the new USBR storage agreement) could theoretically be obtained, but in normal precipitation years the water available in Rapid Creek would be closer to 50 cfs. A quick glance at these numbers indicates that, thanks to the new USBR agreement concerning water rights on Rapid Creek, Rapid City has no immediate water supply problem for the next 30 years. But this does not take into account droughts, peak-use days, or environmental situations such as a gasoline truck spilling into Rapid Creek near Johnson Siding. Complete utilization of Rapid Creek would result in changing a beautiful trout stream flowing through the parkway area of Rapid City into an ephemeral channel below the municipal water intake near Baken Park.

Presently about 10 cfs is being withdrawn from the eight (primarily Madison) wells. The long-term availability of ground water in the western Rapid City area can be estimated as follows: (1) 23 cfs (crude estimate using existing wells pumping continuously), (2) 20 cfs (estimate using recharge rate from
Boxelder, Rapid, and Spring Creeks, giving consideration to other users), (3) 26 cfs (estimate using discharge of existing springs in the Rapid City area). As discussed above, the long-term availability of ground water is in doubt because of the threat of contamination including increases in nitrate due to wastewater systems. Further, there are competing uses for ground water. Probably the most ominous factor that casts a shadow on an increased use of ground water is that the Cleghorn/Jackson Spring complex would undoubtedly be depleted by heavy withdrawals.

Because of the vagaries of estimating long-term availability of ground water, the possibility of contamination of the aquifers, and the effects of large withdrawals on nearby wells and springs, it is recommended that primary reliance be given to Rapid Creek as a future source of municipal water. This water source should provide ample municipal supplies for the next 30 years. The number of municipal wells should not be expanded and withdrawals from the existing eight wells should not be increased.

In the last decade, many municipalities in South Dakota have focused on the Missouri River as a source of water. The Lewis and Clark water system is being constructed to Sioux Falls. The Mni Wiconi Project, currently under construction, will pump Missouri River water from Oahe Reservoir to communities in western South Dakota, including Red Shirt just downstream from Hermosa. In the 1980s, the “West River Aqueduct” was proposed to deliver Missouri River water to Rapid City. In view of the present quantity of good quality water in Rapid Creek, these schemes seem remote. However, water in the Black Hills is finite, and the future needs of Rapid City could ultimately entail the Missouri River.

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SOUTH DAKOTA DIVERSITY OF TEMPERATURE: PICTURES FROM STATISTICAL ANALYSIS

B. A. Shmagin and D. P. Todey
Department of Agricultural & Biosystems Engineering
South Dakota State University
Brookings, SD 57007

ABSTRACT

The regional diversity of monthly temperature was analyzed based on long-term data obtained for South Dakota (SD) from the High Plains Regional Climate Center. Multidimensional statistical methods were used and the principal results presented as a sequence of 2- and 3-dimensional scatterplot pictures depicting the quantitative results.

System hierarchical model of landscape was used for research tasks formulation. Initial matrixes for three research tasks were compiled for the state. The first set of initial matrices of time series \( \{X_{t,n}\} \), where \( t = \) number of years and \( n = \) number of meteorological stations, contains two matrixes: \( X^1_{(67\times29)} \) and \( X^2_{(33\times94)} \). The second set \(-\{X_{t,m}\}\), where \( t = \) number of years and \( m = \) number of months in a year: \( X^3_{(113\times12)}, X^4_{(110\times12)}, \) and \( X^5_{(102\times12)} \). The third set \(-\{X_{n,m}\}\), where \( n = \) number of meteorological stations and \( m = \) number of months in a year, contains two matrixes: \( X^6_{(29\times12)} \) and \( X^7_{(94\times12)} \).

Statistical analysis allowed us to differentiate weather stations by temporal trends and spatial distribution for the time interval 1932-1998. The most variable stations (Brookings, Camp Crook, and Highmore) were determined; their seasonality was described (the most variable months and correlation among months during the year) and their seasonal regime determined. The average annual and monthly temperature distributions were presented for South Dakota based on 29 and 94 stations for the time intervals 1932-1998 and 1963-1995.

This article contains examples from a larger set of results posted on the Web. The full set of pictures with results of completed analysis is presented on the SDSU Weather and Climate Center website at: http://climate.sdstate.edu/publications/article200800001/.

INTRODUCTION

Human activities and sustainability of ecological systems depend at first on the dynamics of climate events: daily, seasonal, and annual sequences of weather. The most used and representative characteristic of climate dynamics is air temperature. There is a huge literature of temperature regime analysis and the many ways to describe it [Ghil et. al., 2002]. The usual way to present a regional temperature is downscaling from global circulation models [Wu and North, 2003; Spak et. al., 2007]. Very few studies deal with real (empirical) temperature
distribution for regions in space and time [Brunet, et. al., 2007]. Our goal in this article is the visual presentation of the temperature regime spatial diversity as a series of scientific pictures based on empirical data for the state of South Dakota, augmented by a few nearby stations in neighboring states.

The general approach of the research presented here is similar to other works of system analysis of climate which seek “… to reduce the numerous space-time degrees of freedom of the climate system to a minimum number of climatic modes that can explain a maximal part of its variability.” [from Dima and Lohmann, 2004]. The mathematical models are developed for air flow in the atmosphere, and also coupling air flows with ocean flows [Lorenz, 1970]. But those are weather systems with timesteps of hours and days [Toth, 1995], whereas we are dealing with climate systems using monthly values of temperatures that depend more upon weather tracks than on individual weather events.

**METHODOLOGY**

In the case of regional research for an area such as SD, we initially have only a general description of the landscape’s cyber model [Krcho, 1978; Krcho, 2003]. The knowledge about behavior of surface air temperature as a part of climate for SD is derived from the statistical structure describing dimensions and variability of analyzed processes. The primary statistical method used to obtain these dimensions from empirical data is factor analysis. Although our analysis is similar to the factor analysis of monthly air temperature for Spain by Brunet and colleagues (2007), the presented methodology is developed as a sequence of steps with statistical methods to describe at first, spatial diversity of SD surface air temperature and then, temporal behavior of the most variable time series.

Methodology for research included the following steps:

- System approach to the territory
- Task formulation for statistical analysis
- Performing the statistical analysis for individual tasks
- Interpretation of results for every task
- Discussion of results for South Dakota.

Average monthly surface air temperatures were regarded as characteristic of stages for the climate system of SD. A system model of the landscape [Krcho, 1978; Krcho, 2003] was employed for research task formulation. The system approach regards the region studied as a cyber system with initial degrees of freedom equal to the number of observation stations. Factor analysis is then used to obtain the model of structure and dimensions of SD from the initial empirical data.

This set of research tasks was first presented as an approach to analyze regional stream runoff [Shmagin, 1997] and then in a more general form for multilevel landscape water balance [Shmagin and Kanivetsky, 2006]. Table 1 presents seven directions of research and use of statistical analysis as groups of research tasks. Three of the seven were chosen for this research:
#1. Identification and mapping of patterns of multi-year annual air temperature variability for a set of stations.

#2. Description of annual variability (dimension for intra-annual process, the most variable months and links with annual values) for air temperature from meteorological stations chosen as typical for territory by results from Task #1.

#4. Description and mapping of regional features of seasonal average values for air temperature.

We analyzed the regional diversity of monthly temperature based on long-term data obtained from the High Plains Regional Climate Center for SD. Maximum visualization was obtained with use of the sequence of 2- and 3-dimensional scatter-plot pictures depicting quantitative results. Statistical analysis was completed and scatter plots were obtained with use of software STATISTICA [StatSoft, 2004].

**Task #1. Identification and mapping of patterns of multi-year annual air temperature variability for a set of stations.**

Initial matrix: \( X_{t \times p} \), where rows: \( t \) – years, columns: \( p \) - time series of annual air temperature. Matrixes of results: \( \{A_{p \times k}\} \) – factor loadings, as dimensions of process (k) and grouping by types of regime (\( p \Rightarrow k \)); \( \{F_{k \times t}\} \) – factor scores, as components for types of regime.

**Task #2. Description of annual variability (dimension for intra-annual process, the most variable months and links with annual values) for air temperature from meteorological stations.**

Initial matrix: \( X_{t \times p} \), where rows: \( t \) – years; columns: \( p \) - time series of monthly and annual air temperature. Matrixes of results: \( \{A_{p \times k}\} \) – factor loadings, as dimensions of process and number of seasons (k), grouping months (p) by seasonal regimes; \( \{F_{k \times t}\} \) – factor scores, as components for seasonal regime. The station’s time series for that group of tasks chosen for territory by results from Task #1. In the data every one obtained from groups of the existing station regime with highest factor loadings was regarded as typical and most variable for the group.

**# 4. Description and mapping of regional features of seasonal average values for air temperature.**

Initial matrix: \( X_{n \times p} \), where rows: \( n \) - stations of air temperature observation; columns: \( p \) – time series of average values of monthly and annual air temperatures. Matrixes of results: \( \{A_{p \times k}\} \) – factor loadings, as dimensional with the number of seasons and structure to relation of months in a season and seasons in a year; \( \{F_{k \times n}\} \) – factor scores, as distributions of stations by aggregation of average monthly air temperatures.

Empirical data of monthly temperatures for SD obtained in tasks 1 and 2 components were plotted with linear and polynomial (the 5th power) trends.
The use of named trends helps visualize the goal to present main differences in charts of the analyzed time series.

To analyze selected time series the model of simplified Fourier analysis [Shmagin, 1992] was applied like:

\[ Y(t) = a + b* t + A_i \cos \left( \frac{2\pi t}{T_i} - F_i \right), \]

where \( a+b* t \) is a linear part of equation; \( A_i, T_i, F_i \) are amplitude, period and phase of \( i \)-cosinusoid. Parameters for this model were developed using special software was used [Shtengelov, 1994].

DATA AND INITIAL MATRIXES

To proceed with these three research tasks, the search for a station with a data record that covered the mutual time interval most completely as possible was completed. Sets of initial matrices were created based on long-term data obtained from the High Plains Regional Climate Center [High Plains, Web). The first set of initial matrices of time series \( \{X_{1n}\} \), where \( t = \) number of years and \( n = \) number of meteorological stations, contains two matrixes: \( X_1^{(67*29)} \) and \( X_2^{(33*94)} \). In the second set with the general form of the matrix as: \( \{X_{1m}\} \), where \( t = \) number of years and \( m = \) number of months in a year, three real stations appear as result of analysis from first group of research tasks. The most variable stations are: Brookings, Camp Crook, and Highmore, - and the initial matrixes for them are: \( X_3^{(113*12)}, X_4^{(110*12)}, \) and \( X_5^{(102*12)} \).

The third group of research tasks with general view of initial matrix - \( \{X_{nm}\} \), where \( n = \) number of meteorological stations and \( m = \) number of months in a year, contains two matrixes: \( X_6^{(29*12)} \) and \( X_7^{(94*12)} \). The first matrix has average values for the time interval 1932 – 1998 and the second one from 1963 – 1995.

For initial matrices from \( X_1 \) and \( X_6 \) locations of 29 stations and also location for 94 stations for matrixes \( X_2 \) to \( X_7 \) presented on Fig. 1 (Table 2).

The distribution of average temperature for two time intervals is shown in Figure 2 as the most simple and elementary attempt of data analysis. The simplest way to present time spatial variance in temperatures is subtracting the average temperatures for the two time intervals (Fig. 2). Here the intervals each have different lengths and have equal intervals to compare the change in average temperatures. Figure 3 presents the difference between average temperatures for the time period 1932-1998 with a 23-year window: 1932-1954 (year in the middle of interval is 1943), 1954-1976 (1965) and 1976-1998 (1987). Results of simple arithmetic show that during the different time intervals, some parts of SD had increasing and decreasing temperatures.

STATISTICAL RESULTS FOR RESEARCH TASKS

Task #1. Identification and mapping of patterns of multi-year annual air temperature variability for a set of stations.
For the matrix $X'_{(67*29)}$ analysis allowed us to obtain a model with three factors reflecting 87% of initial data variability (Table 3). We see three groups of stations. The groups were denoted by stations with loading on one of the factors that were higher than 0.6. Note that on the last station, there are no high loadings with a single factor. We may place all of the 29 stations in planes of those three factors (Figure 4) and see the synchronization of the regime of temperatures. The factor scores present the main regime differences for the three stations groups (Figure 5). We may choose stations with high factor loadings in every group and those with the longest time series of observations, and then regard those as typical stations for a particular group (Fig. 6).

The same analysis for the matrix $X'_{(33*94)}$ that has the most stations with long mutual time interval of observations allowed work with a model inclusive of four factors (Table 4). The model reflects 91% of initial data variability; has four groups related to factors stations, and 14 stations that do not strongly associate with groups. For four groups of stations, the charts of factor scores show the difference in regime for time interval 1963-95 (Fig. 7) and for each group of stations may be chosen the most variable time series for the period of observation 1963-95 (Tab. 4).

**Task #2. Description of annual variability (dimension for intra-annual process, the most variable months and links with annual values) for air temperature from meteorological stations.**

Three stations: Brookings ($X^4_{(113*12)}$), Highmore ($X^4_{(110*12)}$), and Camp Crook ($X^5_{(102*12)}$) - with longest periods of observation and high factor loadings in the factor model obtained for $X'_{(67*29)}$ were chosen as typical for SD during the time interval of 67 years (1932-1998) (Fig. 8). For each of the initial matrixes: $X^3_{(113*12)}$, $X^4_{(110*12)}$, and $X^5_{(102*12)}$ factor model were obtained.

For Brookings ($X^3_{(113*12)}$), work with this station began first as it is the station with the longest period of record of the three named. The factor model has five factors and reflects 61% of the variability of the initial data (Fig. 9). The factor loadings for monthly and annual temperature together show the winter season (Factor 2 - January and February) as most connected to the annual temperature values (Fig. 9 – right part). Factor scores for each season allow comparison of annual and seasonal charts (Fig. 10). Looking at five seasonal charts (Fig. 10), we see that each appear with negative linear trend lines for the period of observations.

Time series analysis was used for annual and seasonal components of air temperature. The simplified Fourier analysis for annual air temperature gave a model that shows a wave approximation of empirical data (Figure 11) and the seasonal component of annual air temperature was not approximated well by the model (Fig. 12).

For the Highmore station, ($X^4_{(110*12)}$) the factor model reflects 61% of initial data variability and presents five seasons of annual temperature regime (Fig. 13). In comparison to the Brookings station’s annual temperature observations, it correlated slightly more with the winter season (Factor Loading - 0.74). Five seasonal charts (Fig. 13) all demonstrate have different appearances of positive
linear trends for the period of observation. The simplified Fourier analysis for annual air temperature gave a model that shows a wave approximation for the empirical data (Fig. 14).

For Camp Crook ($X_2^{(102\times12)}$), the factor model reflects 60% of initial data variability and presents five seasons of annual temperature regime (Fig. 15). In comparison to Brookings and Highmore, the annual temperature correlated better with the winter season (Factor Loading - 0.81). From five seasonal charts (Fig. 15), three have the appearance of positive linear trends for the period of observation (the Factor 1, 2 and 4) and two (Factor 3 and 5) don’t have a visible trend. The simplified Fourier analysis for annual air temperature gave a model that shows a wave approximation for the empirical data (Fig. 16).

# 4. Description and mapping of regional features of seasonal average values for air temperature.

The variability of monthly air temperature distribution over SD obtained as result of the factor analysis of two matrixes: $X_6^{(29\times12)}$ and $X_7^{(94\times12)}$. The first matrix $X_6^{(29\times12)}$ has average values for the time interval 1932 – 1998 and just simple statistics from the initial matrix $X_6$ allows us to see the mean, minimum, and maximum monthly air temperatures for entire territory (Fig. 17). Factor model obtained from this matrix allows significant steps toward understanding the temperature variability. Two groups of months reflect 98% of initial data variability (Fig. 17). The annual air temperature for SD takes place between those seasons in the table of factor loadings and on the scatter plot (Fig. 18). Actual data shown on the scatter plot are four groups of months.

The factor model obtained from matrix $X_7^{(94\times12)}$ allows an understanding of the temperature variability for a shorter time interval but for a higher number of stations from the initial matrix $X_6$. The model has two groups of months reflecting 94% of the initial data variability (Fig. 19). The annual air temperature for the territory of SD takes place between those seasons in the table of factor loadings and on the scatter plot (Fig. 20). The scatter plot shows three groups of months with the number of members from two to seven. The distribution of stations by factor scores posted in a plane of factors (Fig. 20) has few groups with different number of stations.

CONCLUSION AND DISCUSSION

The first that the models presented is the spatial temporal regime of annual average temperatures in SD for the time interval 1932-98. This regime reflects a three dimensional process that may be traced on the territory (Fig. 21). The second factor model from the first research task allowed not only the tracing of the temperature regime on a bigger set of stations (Fig. 22), but also comparison of two time intervals. In both cases, we have low dimensional processes with components of increasing and decreasing temperatures. The directions of these regimes for the time interval 1963-95 of increasing temperatures are located in
two fuzzy areas in the north and west. Decreasing temperatures is also slightly different in two areas occupying southeast, third of SD.

Looking at the factor model results for two time intervals (1932-98 and 1963-95) of spatial time variability of SD annual air temperature (Figs 21 and 22) we recognize common patterns and differences. On both figures temperatures increase in the northern and western parts of the territory and decrease – in eastern and south central. For a longer time interval of mutual observations (1932-98), two factor scores show increase and decrease; for the shorter time interval (1963-95) the tendency is presented in two pairs of factor score charts and stations. To have two tendencies in regional surface air temperature distribution as observed here supports the idea of global distribution of temperature anomalies (Apguez, et. al., 2007). The picture such as the one published by NASA in December 2006 (Fig. 23) presents the details to fully understand the complexity of the surface air temperature's regime origin through interacting processes of global and regional continental levels.

To choose the typical time series for the SD air temperature regime it is better to use a model with a longer interval of observation. The three stations obtained from the model covering the years of 1932-98 for Brookings, Highmore, and Camp Crook help us not just see the difference in regime during the time interval but also see the two interval locations used for analysis on the longest existing time series (Fig. 24). The typical long interval observation station like Brookings may become intermediate in shorter intervals of comparison (Fig. 24).

Patterns of air temperature for all three stations are similar. In all cases, seasonality is weak (cumulative variability – 60 – 61%), reflecting just the main patterns of correlated monthly temperatures (Tab. 5). All three stations have five seasons and the first and second most variable seasons are the summer and winter months for all three stations.

Having five seasonal regime patterns to compare with the annual regime allows for more detailed pictures, which is better than just comparing annual regime over twelve months. For example, the Factor 3 chart model for Brooking has visually the most declining linear trend, after analysis of the trend during the months that create this seasonal pattern (October and November). We may assume that those month’s regimes responsible for annual temperature decline for the period of observation.

Future research to complete the regime picture for the state of SD has to have goals to analyze seasonality of the most variable stations as we see from the model for 1963-1995: Watertown, Castlewood, Marion, and Harrison (Fig. 22).

Results of simplified Fourier analysis for all three time series (Figs 11, 14, and 16) show the significant oscillation components for annual values; and in case of the Brookings station, the completed analysis is not as clear as the seasonal components (Fig. 12).

Use of simplified Fourier analysis provides an equation for an oscillating curve reflecting the main components of the regime. This curve may be used to describe the regime and for forecast. Also, it may be used for finding a conceptual climate model responsible for the oscillations in the monthly surface air
temperature regime. A more complex approach to analyze the hydrological or climatologic time series [Ghil et al. 2002; Golyandina and Zhigljavsky, 2001] doesn't provide so simple a visualization. To illustrate this point, we refer to two articles discussing the analysis and forecast of the same hydrologic time series [Pekarova P., and J. Pekar (2006); Shmagin, Trizna, 1992].

To discuss the obtained results from the oscillation curve for a typical time series for SD, we have to focus not on the period of represented by a cosin function but on the general character of a curve as a sum of those functions. This way we may say that the time series for Brookings is on an interval of existing data that is general declining and expect an increase in temperature during the period 2020-30. In case of Highmore and Camp Crook, we may expect decline of temperatures of those two stations during time interval of 2020-2030.

System analysis of the regime of annual and monthly air temperatures for SD allows us to obtain spatial-temporal variability factor model with known and high (87 and 91%) representation of variability of initial data. Factor scores plotted on annual scatter plots allow visualization of the difference in the regime for groups of stations associated with certain factors. Factor loadings posted on the map demonstrate spatial differences in the regime, and allow selection of the typical station for each group of stations associated with a given factor. The typical station with empirical monthly observations provides the data for factor analysis of seasonality and time series analysis. The typical station also has one more function: to help visualize the big-picture of each individual time interval used for factor analysis (Fig. 24). All together, the factor model and results of analysis of a typical time series present the structure of spatial temporal variability of the monthly temperatures for SD.

The difference in monthly temperature from data of 29 stations is greater for the winter months (Fig. 17). In January, the gap between max and min temperature is 15.3 ºF, for February – 13.3 ºF and December – 11.6 ºF; the smallest difference (5.8 ºF) occurs August, and annual temperature has also difference - 5.8 ºF.

For different and shorter time intervals from 1963-95 (Fig. 19), the min and max range for the state calculated on the average monthly temperatures is also associated with winter (January riches 25.7 ºF), while April through September have two-digit difference. The smallest difference in the monthly temperature range occurred in November, at 8.6 ºF. The range of annual temperature in the state of SD riches 8.3 ºF.

The discussion of ranges of the difference in minimum and maximum monthly temperatures for SD just evokes the need for understanding this kind of variability. This analysis depicts differences in the average temperature for stations (Figs 2 and 3) and we can trace the difference each month trough the territory.

The factor model for the 29 stations over the 1932-98 time interval presents a distribution of monthly temperatures in four seasons (Fig. 18), but those seasons are not usual ones. Winter months create a second factor associating two months from the spring and fall: March and October. May and June have the highest loadings on the first factor - then with slightly lower loadings five remaining months form a separate group. The second model demonstrated similar
grouping of months; except the first factor, formed a week group seven months from April to October.

Two factors from the model create a plane of scores in which the stations may be posted (Fig. 18), but those scores may be used for regression with the annual temperature (Fig. 25). The annual temperature has a coefficient of correlation with Factor 1 equal to 0.69 and with Factor 2 of 0.72; the values are statistically significant for both cases. Putting values of factor scores on a map of SD (Fig. 26) allows us to obtain a distribution not only of average annual temperature as it is traditionally done (Fig. 2) but also integrated seasonally; the station with highest factor scores have higher temperatures for months in the season, and opposite for negative values.

The range in monthly temperatures for the state of SD is higher for time the time interval 1963-95, but the factor model for this period is very much like that during 1932-98. There are slight differences in the first factor group of months: April - October, - seven months as a group, and a longer time interval in June and May have their own group, with five other months remaining. It is significant that the character of distribution of station by factor scores is pretty much the same for both time intervals. We have one main compact group of stations in the lower right part of scatter plot (Fig. 7c group in “a”) and then few groups (with one, two, and up to eleven stations) scattered in the rest of the factor plane (Fig. 27). The 3D scatter plot became more useful for the separation some of groups, such as case “a” and “b” (Fig. 27).

The pictures obtained for two of the factor score distributions of SD and obtained from the model for the time period of 1963-95 (Fig. 8c) are, at first, very similar from model of 1932-98 and, second, are a significant addition to understanding the spatial distribution that a much better recognizable picture with 94 station presents than to that of just 29. Those pictures (Fig. 26 and Fig. 28) have to be considered with two others (Fig. 25 and 27), and from comprising the way regression lines go through the scatter plot, we may surmise that the regression lines have to go through the groups and that the grouping has some underlying conditions in landscapes of SD for better regressions (Fig. 29).

The pictures from this statistical analysis open avenues to analyze the landscape property that underlies the spatial distribution of average monthly air temperatures. The obtained result may have immediate application for:

- developing climatic monitoring network in SD;
- establishing a plan for regional drought research in SD;
- understanding the bigger picture of scaling issues for average monthly and annual air temperature in SD with connection to global and continental temperatures.

The use of the results obtained and the methodology of system analysis of empirical data may be moving in further directions for performing analysis of spatial temporal variability for maximum and minimum monthly air temperature, monthly sum of precipitation, and total monthly snow accumulation. Results of all named characteristics will open a quantitative landscape regional-
ization for the SD. The straight one-step approach of cluster analysis for regionalization from station’s 22 climatic characteristics [Bunkers, et. al. 1996] does not give diverse understanding and visualization of all components of regional climate process origin.

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### Table 1. Groups of research tasks and statistical methods for multilevel system analysis of climate characteristics of landscape.

<table>
<thead>
<tr>
<th>Group of tasks</th>
<th>Research level</th>
<th>Initial matrix ( X_{\text{row},n} )</th>
<th>Statistical method</th>
<th>Matrices of results</th>
<th>Final graphics and equations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Identification and mapping of patterns of multi-year annual regime variability (stream runoff, air temperature, precipitation) for set of watersheds or stations</td>
<td>Global Regional Basin</td>
<td>Years</td>
<td>Time series (TS) of discharge ( {Q_j} ), temp. ( {T_j} ), and ( {W_j} ) - precip.</td>
<td>Factor, Time series and Cluster analyses</td>
<td>( A_{hq} ) - dimensions of process, grouping by types of regime. ( F_{h} ) - components for types of regime. Ed - distances for watersheds and observation years.</td>
</tr>
<tr>
<td>2. Description of annual variability (dimension for intra-annual process, the most variable months and links with annual values) for runoff from watershed, ground water level (GWL) in wells and data from meteorological stations, trend analysis</td>
<td>Planet Global Regional Basin Station</td>
<td>Years</td>
<td>TS of discharge ( {Q_{j,12,13}} ), level ( {H_{j,12,13}} ), temperature ( {T_{j,12,13}} ), and precip. ( {W_{j,12,13}} )</td>
<td>Factor, Time series and Cluster analyses</td>
<td>( A_{hq} ) - dimensions of process, grouping by seasons regime. ( F_{h} ) - components for seasons. Ed - distances for months and observation years</td>
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<td>3. Establishment of association between multi-year runoff parameters and other state indices or attributes of landscape</td>
<td>Planet Global Regional Basin</td>
<td>Years</td>
<td>TS of discharge ( {Q_j} ), and state indices ( {H_j} )</td>
<td>Factor analyses and Step by step regression</td>
<td>( Y=a_0+\sum a_i x_i + e ), - regression equation</td>
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<td>4. Description and mapping of regional features of seasonal average values for runoff, GWL and meteorological data</td>
<td>Global Regional Basin</td>
<td>Watersheds. Stations or well of observation.</td>
<td>Average values of runoff TS ( {Q_j} ), ( {Q_{j,12,13}} ), and meteorological data TS ( {T_j} ), ( {W_j} )</td>
<td>Factor analyses</td>
<td>( A_{hq} ) - structure of relations. ( Y=a_0+\sum a_i x_i + e ), - regression equation</td>
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<td>5. Identification of relationship between surface and GW runoff parameters, min and max temperatures</td>
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<td>Watersheds</td>
<td>Runoff parameters ( {q_j, k_j} )</td>
<td>Factor analyses</td>
<td>( A_{hq} ) - dimensions of process and structure of relations. ( F_{h} ) - grouping of watersheds by generalized characteristics</td>
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<td>6. Establishment of relationship between runoff parameters distribution and attributes of atmosphere and lithosphere components for watersheds</td>
<td>Regional Basin</td>
<td>Watersheds</td>
<td>Parameters of runoff and attributes of atmosphere and lithosphere conditions ( {q_j, k_j, T_{12}, W_{12}, H_{12}} )</td>
<td>Factor analyses and Step by step regression</td>
<td>( Y=a_0+\sum a_i x_i + e ), - regression equation.</td>
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<td>7. Reevaluation and mapping of units with quazi-uniform landscape conditions (elements of regionalization), reevaluation of the influence on river runoff components (ground water and surface)</td>
<td>Regional Basin</td>
<td>Watersheds</td>
<td>Parameters of runoff and attributes of atmosphere and lithosphere conditions ( {q_j, k_j, H} )</td>
<td>Factor analyses, Step by step regression, Student, Fisher and Nonparametric tests</td>
<td>( A_{hq} ) - structure or runoff parameters relations by elements of regionalization. ( Y=a_0+\sum a_i x_i + e ), - regression equation.</td>
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Table 2. List of station with mutual time interval 1963-95 (94 stations – Nº 1) and 1932-98 (29 – Nº 2), YMMD – years with missing monthly data, % - percent of YMMD to years in the time interval.

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<th>Nº2</th>
<th>Name</th>
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<th>Latitude</th>
<th>Elev [m]</th>
<th>YMMD</th>
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Table 3. Factor Loadings for 29 stations for mutual period of observations 1932-98 (shown values > 0.25).

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Explained Variability 10.50  7.31  7.65
Proportion of Total 0.36  0.25  0.26
Table 4. Factor Loadings (left – sorted by number, right – presented by groups) for 94 stations for mutual period of observations 1963-95.

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Camp Cr. 0.81 0.33 0.32 0.34
Figure 1. Coop meteorological stations from HP Web site with numbers of rows in initial matrixes for: 1932-98 time interval and 29 stations (upper); 1963-1995 (94) – (lower). Size of sign follows grades in elevation [m]: 354, 466, 631, 850, 1128 & 1631.
Figure 2. Annual average air temperature for: 1938-98 (29 stations) - top; 1963-1995 (94) - bottom; the difference in temperature for 29 station (middle). Size of sign follows grades in temperature [F] for top: 42, 44, 46, 48 and 49.9; for bottom: 41.4, 43.9, 45.7, 47.3 and 49.7 and for the middle: -0.31, 0, 1, 2, and 2.44.
Figure 3. Difference in average annual air temperatures [°F] for period 1932-98 with window 23 years: 1932-1954 (year in the middle of interval is 1943), 1954-1976 (1965) and 1976-1998 (1987). Size of sign follows grades in temperature [°F]: 0.0 – 1.0; 1.0- 2.0 and > 2.0. The red color of sign shows increase and blue – decrease in temperature for interval: a- 1943-65; b- 1943-87 and c- 1965-87.
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Figure 10. Seasonal structure of annual temperature for Brookings (from Table 5) and charts of annual and seasonal regime.

Figure 11. Annual air temperature for Brookings (1893-2005) and results from simplified Fourier analysis (Table). Blue line - annual temperature, black lines are linear and polynomial trends, pink line is model composed from cosins with parameters from Table.
Figure 12. Charts of annual temperature and seasonal components for empirical data and models from simplified Fourier analysis (Tables). Blue line - annual temperature and seasonal components, black lines are linear and polynomial trends, pink line is model composed from cosinuses with parameters from the Table.
Figure 13. Seasonal structure of annual air temperature for Highmore (from Table 5) and charts of annual and seasonal regimes.

Figure 14. Annual air temperature for Highmore (1904-2005) and results from simplified Fourier analysis (Table). Blue line - annual temperature, black lines are linear and polynomial trends, pink line is model composed from cosinuses with parameters from the Table.
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Figure 16. Annual air temperature for Camp Crook (1896-2005) and results from simplified Fourier analysis (Table). Blue line - annual temperature, black lines are linear and polynomial trends, pink line is model composed from cosines with parameters from the Table.
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Figure 18. Table of Factor Loading and scatter plots for monthly air temperature for territory of South Dakota (upper part); scatter plot for Factor Scores from 29 stations for 1932-98 (down).

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Figure 19. Statistics and factor model for monthly air temperature for the state of South Dakota: left – graph and table from 94 stations for 1963-95; right – scatter plot and table for Factor Loading.
Figure 20. Table of Factor Loading and scatter plots for monthly air temperature for territory of South Dakota (upper part); scatter plot for Factor Scores from 94 stations for 1963-95 (down).

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Figure 21. Distribution of Factor Loadings for period of observation 1932 – 1998; size of sign follows grades in factor loadings: 0.25 – 0.5; 0.5-0.7; 0.7- 0.99, charts of annual temperature regime (°F) for three the most typical for every group of stations; the box shows the mutual interval; charts of factor scores.
Figure 22. Distribution of Factor Loadings (middle) for period of observation 1963 – 1995; size of sign follows grades in factor loadings: 0.25 – 0.5; 0.5-0.7; 0.7- 0.99 and charts of factors scores (left) and of annual temperature regimes (°F) for four of the most typical group of stations (right): Watertown, Castlewood, Marion and Harrison, NE.
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Figure 28. Distribution of Factor Scores for stations during the observation period of 1963 – 1995; size of signs follows grades of negative and positive Factor Scores values: < -1.0; -1.0 – 0.0; 0.0 – 1.0; > 1.0.
Figure 29. Regression of annual temperature from Factor Scores for stations for 1932-98 (upper) and 1963-95 (lower). The regression lines inside the ovals (as the long axis) have to provide a higher coefficient of correlation.
OFF-SITE IMPACTS FROM ABANDONED URANIUM MINES IN THE NORTH CAVE HILLS, HARDING COUNTY, SOUTH DAKOTA

Larry D. Stetler
Department of Geology and Geological Engineering
SD School of Mines and Technology
Rapid City, SD 57701

James J. Stone
Department of Civil and Environmental Engineering
South Dakota School of Mines and Technology
Rapid City, SD 57701

Albrecht Schwalm
Medford, OR

ABSTRACT

Mining of uraniferous lignite in the Tertiary Fort Union Formation occurred in South Dakota from 1954 through 1967. Mining law required no site remediation on federal land resulting in ~40 years of environmental exposure at these locations. Extent and location of off-site contamination has been largely unknown. A Joint Venture Agreement between the USDA-Forest Service Northern Region and the South Dakota School of Mines through US-EPA CERCLA funding was established to determine offsite impacts from contamination in NW South Dakota. Watershed and airshed level analyses indicate significant and widespread contamination of heavy metals and radionuclides on private properties. Contamination occurs through transport and deposition of sediments containing As, Cu, Mo, Ra\textsuperscript{226}, Th, U, and V. Concentrations of As, Cu, Mo, U, and V exceeded established background concentrations within several streams below mine sites with total metal concentrations exceeding 35 times established background levels. Surface-water contamination was limited to ~27 km of stream length below abandoned mines. Seventy-six percent of ground-water wells contained gross alpha emitters with three wells above the maximum contaminant level (MCL) for drinking water (15 pCi/L). U, U\textsuperscript{235}, Ra\textsuperscript{226}, Se, Cu, Mo, Pb, and Zn also were present at low levels. Although surface-water data and airshed analyses indicated contamination on private properties, no data indicated ground-water composition was adversely affected by mining. Wind-derived dust contained V, Cu, As, Pb, and Th in all of the 34 samples. U occurred in 32 samples and was above established background (0.74 mg/kg) in 15 samples. Distribution and occurrence of these metals indicate they were natural components in the dust. Only U concentrations occurred in the direction of the predominant wind decreasing to background within 15 km of the mine sites.
Keywords
Uranium, Reclamation, Mining, CERCLA

INTRODUCTION

Uranium exploration in northwestern South Dakota began in 1954 when the Atomic Energy Commission planned to fly airborne surveys over the Slim Buttes. Weather conditions precluded flying the Slim Buttes and instead, the survey was flown over the North Cave Hills (NCH) (Curtiss, 1955). As a result, the first claims were staked in the NCH on August 15, 1954 and mining ensued that year. Mine sites were located primarily within an approximately two mile wide northwest trending strip crossing the central NCH, referred to as the hot zone. Mining was permitted under the General Mining Laws and Public Law 357, which required no form of restoration. Most mines and mining prospects were located on United States Forest Service (USFS) administered land, but at least two actively mined sites and several prospects and exploration cuts and digs were located on private land surrounding the NCH. Most of the uranium mines were abandoned coal strip mines located on relatively flat areas along the top of the numerous buttes that characterize the area. Mining consisted of the removing overburden (up to 80 feet) to reach the ore zone which consisted of uranium-bearing lignite beds. Mining activity increased through the next decade but ceased altogether in 1964.

During mining, most of the overburden and mine spoils were pushed over the edges of the buttes where subsequent erosion has spread materials over almost 1,000 acres in the NCH alone. Since mining ceased, additional deposition has occurred down-slope onto private land by water and wind transport. The bluffs and slopes immediately below mine sites often were covered by spoils forming highly over-steepened talus slopes, several of which have failed or are deeply incised. Currently, spoils are mostly devoid of vegetation and their composition has promoted water channeling, gully ing, and tunneling. In addition to mined sites, numerous prospecting pits or contour benches were excavated on both USFS and private land and have been mapped and documented (Pioneer, 2005). Characterization of the abandoned mine sites on USFS land has occurred (Pioneer, 2005; Portage, 2005) but no work had characterized off-site impacts on surrounding private land. A Joint Venture agreement between the United States Department of Agriculture-Forest Service and the South Dakota School of Mines and Technology (including a subcontract with Oglala Lakota College) was established in 2006 to address potential off-site impacts. Funding for this on-going study has been provided through US-EPA CERCLA. Objectives of the NCH project were to determine whether heavy metal and radionuclide environmental contaminants have been transported from historical mine sites located on USFS land onto surrounding private land. Mechanisms of environmental transport most likely include the following:
- erosion of spoil sediments through surface runoff into adjacent drainages
- erosion of spoil sediments through slope failures below mined bluffs
- dissolution of hazardous metals within runoff water that form tributaries to streams
- erosion/deposition of small particle contaminants by wind transport
- infiltration of hazardous metals and radionuclides into local and regional ground water aquifers

**METHODS**

Target analytes for metals and radionuclides and analytical methods and procedures for determination of analyte concentration were similar to those used in previous investigations (Pioneer, 2005; Portage, 2005). Metals of interest included: U, As, Se, Cu, Mo, Pb, Th, and V. Water samples were further analyzed for gross alpha radiation, Ra$_{226}$, and U$_{235}$.

A watershed approach was developed to discern potentially impacted surface waters. It was assumed that all runoff water and eroded sediments (except for wind erosion) would ultimately end up in adjacent drainages and subsequently migrate downstream through established drainage networks. Surface-water sampling events were divided into two separate phases. Initial Phase I sampling evaluated target analyte concentrations of all potentially impacted drainages at the USFS/private land boundary. Phase I data also were used to establish background concentrations based on sampling locations assumed to be unaffected by mining activities. These data were used subsequently to evaluate which drainages were most heavily impacted. After evaluation of the Phase I results, drainages with the highest environmental concern were selected for Phase II sampling which continued downstream to a point where target analyte concentrations were comparable to established background levels.

Ground-water quality was evaluated by selecting 34 wells that were evenly distributed around the NCH within eight km of the USFS boundary. Approximately half of the wells were domestic supply wells and the others were stock wells. Well depths ranged between 10 and 240 m and represented shallow unconfined alluvial aquifers and deep confined aquifers. Samples were collected after pumping a volume of water equal to or exceeding two times the casing volume.

Airborne dust particulates were collected from 30 locations in two sampling phases. Phase I sampling occurred within the set 8 km radius from the USFS boundary. These data were used to establish background concentrations in regions where impacts from wind were assumed to be negligible. Phase I results were utilized to identify directions and regions to obtain additional samples in Phase II until a reduction in concentrations were observed. Airborne dust particulates were collected using a portable wind tunnel (Stetler, 1999) following these criteria:
sites were high elevations above stream courses to ensure dust was either deposited directly by air or was derived by soil-forming processes
- sampling sites were designed in a ‘gridded’ network around the NCH and were sufficiently distributed in accordance with the prevailing wind directions
- sites were at locations that had physical indications of the presence of fine-grained surface materials derived from mechanisms excluding fluvial processes
- locations had surfaces where dry and loose fine-grained materials were readily available to wind processes and could be collected either from the existing surface or generated through preparation of a standard loose surface (Saxton et al., 1998)

Additional dust samples were collected using a stainless steel soil scoop to skim the extreme upper layer of soil from the surface in areas inaccessible to the wind tunnel.

Samples collected to represent potential environmental transport by the above listed mechanisms were compared to established background values calculated using:

\[
\text{mean} + 3 \times \text{standard deviation}
\]  

These values were determined using samples collected from non-impacted drainages and represent natural metals concentrations. For airborne dust particulates, a background value of mean + 2 × standard deviation was used.

RESULTS

The key metals of interest in this study were U and As, although concentrations for the full suite of analytes listed above were determined from each sample. A complete listing of all metals and detailed discussion of trends and occurrences were given by Stone et al. (2007) or can be downloaded at http://uranium.sdsmt.edu. In the following text, only U and As results will be presented.

Soils

Soil samples were collected from cores (0.3—8 m deep) drilled in numerous watersheds surrounding the mined and non-mined areas. Two or more composite samples were prepared from most of the deeper cores. Shallow cores yielded a single sample. Background concentrations were determined using sites representing pristine environments not affected by mining.

Uranium concentrations in soils were mostly below the background value of 22 mg/kg and had a generally decreasing trend with increasing distance from the source areas (Stone et al., 2007). Higher values were associated with washover deposits and channeling of sediments. Values up to 2× background were located immediately below Bluff B (Fig. 1).
Arsenic concentrations followed a trend similar to U concentrations. Background concentration was 32 mg/kg and was exceeded at several locations at values up to 64 mg/kg. The highest As value (96 mg/kg) was at the site with the highest U concentration.

Results showed that 14 watersheds were potentially impacted by sediment transport from previous mining activity. The most impacted area was in the Upper Pete's Creek drainage below Bluff B where two U samples were 3× and 4× of background. All other U concentrations were below 2× background.

Figure 1. Location map of the North Cave Hills, NW South Dakota, showing the mined Bluffs designated by the cross-hatch pattern and labeled A-L. Outline shape represents the US Forest Service boundary. Locations of Upper Pete's Creek and Schleichart Draw are shown and are discussed in the text.
Surface Water

Fourteen watersheds were identified within the study that were potentially impacted by uranium mining. Four pristine watersheds were used to determine background concentrations for all analytes. Sampling of surface water was completed in two phases; Phase I was a large-scale attempt to define contamination regionally, and Phase II sampling isolated impacted areas and delineated contaminant extent. Results indicated there were two impacted areas: the Upper Pete’s Creek watershed below and east of Bluff B where surface waters were derived from direct runoff of the spoils piles, and the Schleichart Draw which received runoff down the western slopes of the North Cave Hills.

Phase I sampling indicated elevated U and As concentrations in both watersheds. Elevated U concentrations in the Upper Pete’s Creek drainage ranged from 2.9× to 5× above the background value of 0.027 mg/L. Elevated As concentrations ranged from 28× to 33× above the background value of 0.020 mg/L. In Schleichart Draw, elevated U concentrations were 3.6× background. These results were used to define Phase II sampling to isolate the contamination and identify locations with the greatest contamination.

Phase II sampling indicated severe contamination in the Upper Pete’s Creek watershed below and to the east of Bluff B where the highest U and As concentrations were 23× and 89× background, respectively. Sampling in the downstream direction showed that natural attenuation of contaminant concentrations has occurred within a distance of ~5 km below Bluff B. Below-background values were observed for all analytes at the confluence of Pete’s Creek and Crooked Creek, ~ 15 km northeast of Bluff B.

Ground Water

Contaminant occurrence was ubiquitous in ground-water samples at all depths of wells tested and was distributed within the study area both up and down gradient. The exceptions were thorium, arsenic, and vanadium, which were not detected in any samples. Trends of occurrences suggest that metals detected were regional, naturally occurring contaminants and not directly attributable to uranium mining.

Water quality was compared against maximum contaminant levels (MCLs) established by the US EPA (US EPA, 2006). Uranium was detected in seven wells and ranged from 0.001 to 0.064 mg/L. One well was 0.064 mg/L and exceeded the MCL of 0.03 mg/L by a factor of two. This well was 18 m deep and was fed by shallow alluvial gravels with hydraulic connectivity to uraniferous lignites updip from the well. A second well contained a U concentration of 0.027 mg/L and was also a shallow well in alluvium.

Many elements in the U decay series can contribute radionuclide counts in a sample. Analysis to determine which element contributed what amount of radioactivity is expensive and time-consuming. Thus, gross alpha counts were used to assess the radioactivity of the sample. The EPA has established a MCL of 15 pCi/L for gross alpha. Twenty-six of the 34 wells contained gross alpha radiation ranging from 1.8 to 44.4 pCi/L. Distribution of these wells were up and down
gradient and at depth from 18 to over 240 m. Three wells exceeded the MCL for uranium. The two highest concentrations were the shallow wells discussed above and the third highest concentration was a 122-m-deep well completed into the Fox Hills sandstone located northeast of Bluff B.

In addition to U concentrations and gross alpha contents, three wells contained Ra$^{226}$ in concentrations ranging from 0.5 to 0.7 pCi/L and were all well below the MCL of 5 pCi/L. Two of the wells had concentrations of 0.7 pCi/L and were the same wells as those with the highest gross alpha contents discussed above. U$^{235}$ was detected in five wells at concentrations between 0.3 and 1 pCi/L, up and down gradient of the mine sites. There is no MCL for U$^{235}$.

Data collected and analyzed during the ground-water study indicate that metals and radionuclides were natural components of the ground-water systems. Further, the distribution of the contaminants shows metals were dissolved between the recharge areas to the west and the North Cave Hills. It is not clear if the abandoned uranium mines in the North Cave Hills contribute directly to the metals content of the ground water. Most likely the chemistry of surface water and local springs were affected by the mines but the deep aquifers should not be affected directly. Shale confining layers above the deep sources theoretically protect infiltrating waters from reaching these aquifers. The exception would be the presence of deep fracture systems allowing local infiltration to reach the water table, i.e., a leaky confining layer. These conditions will be evaluated in future studies.

Aerosols

Results of the surface dusts indicate the general ubiquity of target analytes in the soils around the North Cave Hills. Uranium was present in all but two samples and arsenic was present in all samples. Vanadium, copper, and thorium also were present in all samples and lead occurred in all but one sample. Molybdenum and selenium had the least occurrences.

Thirteen samples contained uranium concentrations in excess of the calculated background value of 0.74 mg/kg. Distribution of these sites extended across the entire sampling area and were classified into three distinct domains:

1) Eight locations, including the three with the highest uranium concentrations (1.96, 1.66, and 1.6 mg/kg), occurred in a northwest to southeast trend cutting across the center of the North Cave Hills and containing the largest abandoned uranium mine areas. The long axis of this high-concentration area also correlated to the predominant wind direction, indicating a probable wind influence on the observed distribution. The greatest uranium concentration (1.96 mg/kg) occurred on the western side of the North Cave Hills below and upwind of Bluffs J-K and upwind of all of the abandoned mine sites.

2) Two locations north of the North Cave Hills had uranium concentrations greater than 1.0 mg/kg and both were in a direction of minimal above-threshold wind occurrences.
3) Two locations in a topographic low between the North and South Cave Hills (Bull Creek drainage) had uranium exceedences. Uranium concentrations were below background levels in all areas away from these three identified areas of high concentrations. Arsenic was detected in all samples and five contained concentrations above the calculated background value of 11.93 mg/kg. All exceedence concentrations were east and south of the North Cave Hills. However, concentrations below the background level that were between 9.8 and 11.6 mg/kg were located near the exceedence locations and appeared to form a similar northwest to southeast pattern across the center of the North Cave Hills as was noted for uranium domain 1. Two locations in the north were close in value to the As background level that corresponded to uranium domain 2. Thus, there is some evidence for wind distribution of fine particles that contained these metals.

DISCUSSION

Significant environmental degradation has occurred from transport of heavy metals and radionuclides downstream of abandoned uranium mines in the North Cave Hills. The most impacted regions occurred down gradient of Bluff B and in Schleichart Draw in the SW of the study area. Surface water contained U and As up to 90× background values and sediment had concentrations 4× background levels. Metals in sediments and surface water were naturally attenuated within ~15 km below the mine sites, although large deposits of contaminated soils occurred on private lands. Ground water contained many metals and radionuclides in both the up and down gradient directions and at shallow to deep aquifer depths. This indicated that metals contamination was regional in extent and most likely were not affected by U mining. Aerosol dust had significant metals concentrations in nearly all locations sampled. Uranium was ubiquitous and contained at least one plume of high concentrations that correlated to the predominant wind direction. All metals concentrations in aerosols were decreasing or below background levels within about 15 km from the mine sites. Although U, As, and other metals were detected in aerosols, their concentrations were on average seven times less than metals concentrations in soils, indicating aerosol transport of metals remains low.

LITERATURE CITED


MOLECULAR COMPARISON OF FIVE FHB RESISTANCE SOURCE IN WHEAT USING SSR MARKERS

Yuejin Weng and Yang Yen
Department of Biology/Microbiology
South Dakota State University
Brookings SD, 57007

ABSTRACT

Five FHB-resistant wheat lines (Abura, N4780, Sumai 3, Tokai 66, Wanshuibai) were genotyped with 80 SSR markers, which cover all of the 42 chromosome arms of the bread wheat genome. A total of 267 allelic variants were detected at the 80 SSR loci, ranging from one to ten per locus with an average of 3.34. The genetic distances between the five cultivars were estimated with UPGMA. Our analyses grouped Abura with Tokai 66 and Sumai 3 with Wanshuibai, respectively, leaving Ning 7840 standing distantly from them.

Key words

Fusarium head blight, molecular genetic analysis, Triticum aestivum L.

INTRODUCTION

Fusarium head blight (FHB) caused by Fusarium graminearum, is a destructive disease in wheat and barley. Production losses due to FHB can range from 30-70% where conditions favor the disease. FHB-infected grains contain mycotoxins, such as trichothecenes deoxynivalenol and nivalenol can cause health problem when they are consumed. Development and utilization of resistance wheat varieties is the most economic, efficient, and environment friendly measure for controlling the disease. Several FHB-resistance sources have been identified. Knowledge of their genetic backgrounds is needed before they will be used in breeding programs to avoid redundancy in breeding efforts. Molecular markers provide a means to obtain such information. This communication reports our efforts to get such information with the aid of short sequence repeat (SSR) markers.

MATERIALS AND METHODS

Plant Materials

Wheat cultivars used in this study are listed in Table 1. The five FHB-resistant cultivars were reported among the best FHB sources. Particularly, Sumai
3 is the most used FHB resource in breeding for better FHB resistant in the world. Two FHB-susceptible lines (Wheaton and Y1193) were used as checks in our field validation of FHB resistance. Field validation of FHB resistance of the cultivars used was conducted in a mist-irrigated nursery at the Agronomy Farm at the campus of South Dakota State University campus in Brookings, SD, USA in 2005.

Each field plot consisted of two 0.15M-by-1.0M rows. *Fusarium graminearum* isolate Fg4 was used to induce FHB. The conidiophores inoculum was prepared with potato dextrose agar plates and suspended into deionized water. Corn kernels inoculated with *F. graminearum* for at least one week were spread in the field weekly starting from the jointing stage to the end of anthesis. Also, water suspension of *F. graminearum* conidiophores (approximately $10^5$ microconidia/mL) was sprayed to the wheat spikes first at anthesis and then one week after the initial inoculation. A mist-irrigation system was used to maintain humidity in the field.

Disease incidence (DI, as percentage of scabby spikes per plot) was visually estimated two weeks after the first spray inoculation. The *Fusarium*-damaged kernels (FDK) were counted after the heads were harvest. Kernels were defined as “tombstone” if they appeared to be shriveled or discolored. FHB-diseased kernel (FDK, as percentage of tombstone kernels) was recorded by visually examining 103 randomly picked kernels per line.

**SSR marker assay**

SSR markers used in this study were listed in Table 2. They were selected due to their genomic locations. PCR primers for these SSR markers were synthesized by Invitrogen Inc. according to sequence information published by Roder et al (1998) for the gwm series and to USDA-ARS Beltsville Agriculture Research Center for the BARC series.
Genomic DNA were extracted from 2-cm long pieces of young leaf tissues sampled from greenhouse-grown plants with DNAzol by following the procedure provided by the manufacturer Molecular Research Center, Inc. PCR amplification was done as described by Roder et al (1998). Reaction was performed in a final reaction volume 20 ml in 0.2ml Eppendorf tube. The reaction mixture contained 50-100ng of template DNA, 2uM primers (R and F), 0.5U Taq polymerase, 50mMdNTP, 1×PCR buffer, and 1.5mM MgCl₂. After 3 min denaturation at 94ºC, 45 cycles were performed with 1 min at 94ºC, 1 min at 50ºC, 55ºC, 58ºC, or 60ºC (primer dependent), 2 min at 72ºC, and a final extension step of 10 min at 72ºC. PCR products were separated on denaturing gel with 6% agarose.
polyacrylamide. Electrophoresis was performed with 0.5×TBE buffer with 2KV for 1 hour and visualization by silver staining as described by Yen et al. (2000). The size of an allele was determined through comparison with a 10-bp DNA ladder included on all of the gels. Absence of a marker from an entry was scored as “0” and its appearance was scored as “1”. Genetic distance was determined by Genetic Distance Method=UPGMA modified from NEIGHBOR procedure of PHYLIP Version 3.5.

RESULTS AND DISCUSSION

The results of the field validation

The field performance in 2005 was listed in Table 1. The five FHB-resistant cultivars differed in many agronomically important traits when grown at Brookings, SD (Table 3). Our field trial validated previously reports about the FHB resistance/susceptibility of all of the entries. Wangshuibai performed the best in our trial with 10% DI and 6% FDK (Table 3).

The results of SSR genotyping

A total of 267 alleles were scored for the 80 SSR markers assayed with an average 3.34 alleles per marker. Alleles for each SSR marker were listed in Table 4. Primer sets gwm16, gwm68, gwm192, gwm219, gwm333, and gwm573 only revealed one allele each, but primer sets gwm126 gwm47 and gwm55 each revealed 10 alleles.

To elucide genetic similarity/difference among the five FHB-resistant cultivars, genetic distance was estimated according to Nei (1978). The results were shown as a dendrogram in Fig 1. The genetic distance between the entries varied from 17.01 (between Sumai 3 and Wangshuibai) to 30.15 (Ning7840 to the other four). The UPGMA clustering clearly grouped Abura with Tokai 66 and Sumai 3 with Wangshuibai, respectively. Ning 7840 was found to be

<table>
<thead>
<tr>
<th>Accession</th>
<th>Plant high (cm)</th>
<th>Awn</th>
<th>Vernalization</th>
<th>Anthesis (Day)</th>
<th>FHB-RESISTANCE</th>
<th>DI</th>
<th>FDK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abura</td>
<td>77—87</td>
<td>No</td>
<td>Spring</td>
<td>86</td>
<td>40%</td>
<td>15%</td>
<td></td>
</tr>
<tr>
<td>Ning 7840</td>
<td>47—57</td>
<td>Mediate</td>
<td>Spring</td>
<td>86</td>
<td>40%</td>
<td>15%</td>
<td></td>
</tr>
<tr>
<td>Sumai 3</td>
<td>57—67</td>
<td>Mediate</td>
<td>Spring</td>
<td>84</td>
<td>40%</td>
<td>18%</td>
<td></td>
</tr>
<tr>
<td>Tokai 66</td>
<td>59—69</td>
<td>Mediate</td>
<td>Spring</td>
<td>82</td>
<td>40%</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td>Wangshuibai</td>
<td>62—72</td>
<td>Long</td>
<td>Mediate spring</td>
<td>95</td>
<td>10%</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>Wheaton(CK)</td>
<td>57—67</td>
<td>Long</td>
<td>Spring</td>
<td>80</td>
<td>60%</td>
<td>99%</td>
<td></td>
</tr>
<tr>
<td>Y1193(CK)</td>
<td>69—79</td>
<td>No</td>
<td>Mediate spring</td>
<td>100</td>
<td>70%</td>
<td>70%</td>
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</tr>
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</table>
Table 4. SSR markers linkage with FHB resistance genes and QTL among five FHB resistance sources in spring wheat.

<table>
<thead>
<tr>
<th>Population</th>
<th>Abura</th>
<th>Ning 7840</th>
<th>Sumai 3</th>
<th>Tokai 66</th>
<th>Wangshuibai</th>
<th>BARC15</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>220;250</td>
<td>220;250</td>
<td>220;250</td>
<td>220;250</td>
<td>220;250</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gwm425</td>
<td>140</td>
<td>170</td>
<td>140</td>
<td>135</td>
<td>135</td>
</tr>
<tr>
<td></td>
<td>Gwm296</td>
<td>140</td>
<td>140</td>
<td>140</td>
<td>133</td>
<td>Ning 7840/Freedom</td>
</tr>
<tr>
<td></td>
<td>Gwm120</td>
<td>128</td>
<td>140</td>
<td>140</td>
<td>140</td>
<td>Ning 7840/Clark</td>
</tr>
<tr>
<td></td>
<td>BARC91</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>W14(from Sumai 3)/Madison</td>
</tr>
<tr>
<td></td>
<td>Gwm539</td>
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<td></td>
<td>Gwm369</td>
<td>165</td>
<td>165</td>
<td>165</td>
<td>165</td>
<td>Wangshuibai/Falat</td>
</tr>
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<td></td>
<td>Gwm285</td>
<td>240</td>
<td>220</td>
<td>230</td>
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<td>240</td>
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<tr>
<td></td>
<td>Gwm389</td>
<td>140</td>
<td>140</td>
<td>115</td>
<td>140</td>
<td>136</td>
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<tr>
<td></td>
<td>Gwm495</td>
<td>178</td>
<td>178</td>
<td>178</td>
<td>178</td>
<td>160</td>
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<tr>
<td></td>
<td>Gwm126</td>
<td>200;210;230</td>
<td>200;210;230</td>
<td>192;202;222</td>
<td>195;205;225</td>
<td>192;202;222</td>
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<tr>
<td></td>
<td>Gwm293</td>
<td>130;138</td>
<td>130;138</td>
<td>130;138</td>
<td>130;138</td>
<td>130;138</td>
</tr>
<tr>
<td></td>
<td>BARC239</td>
<td>140</td>
<td>140</td>
<td>140</td>
<td>140</td>
<td>140</td>
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<tr>
<td></td>
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<td>125</td>
<td>124</td>
<td>125</td>
<td>125</td>
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<td>BARC1096</td>
<td>140;148</td>
<td>150;158</td>
<td>150;158</td>
<td>150;158</td>
<td>150;158</td>
</tr>
<tr>
<td></td>
<td>Gwm 46</td>
<td>145;155</td>
<td>165;180</td>
<td>165;180</td>
<td>145;155</td>
<td>138;145</td>
</tr>
</tbody>
</table>

Figure 1. Dendrogram based upon Nei’s (1978) genetic distance among the five sources.
genetically distant from the other four. This UPGMA clustering indicated that the five FHB-resistant sources differed from each other. Breeders should take into account genetic traits into breeding in order to pyramid different superior agronomic traits with FHB breeding in wheat.

REFERENCES


A STUDY OF PROMOTER ELEMENTS OF HOMO SAPIENS

Yunkai Liu, Sujuan Ye and Asai Asaithambi
Department of Computer Science
University of South Dakota
Vermillion, SD 57069

ABSTRACT

Promoters are essential functional units of gene transcription. The discovery of combinational patterns of promoter elements is a challenge problem in computational biology. In this paper, we studied promoter elements (PEs) in extended core promoter regions of homo sapiens using a binary matrix representation. Our study revealed many new interesting patterns of occurrence or non-occurrence of these PEs. Sp1 and Inr are the most commonly occurring PEs. We observed that, two different PEs do not occur simultaneously, and the same PE does not occur in different windows simultaneously. The set of PE-Window combinations, which we call features, lends itself to a partitioning into two subsets such that the presence of any feature in one set implies the absence of any feature in the other. Communities characterized by high occurrences of a select set of features exhibit some significant properties with respect to the presence or absence of other features.

Key words

Core promoters, promoter elements, features, binary representation, communities

INTRODUCTION

Promoters are essential functional units of gene transcription that integrate all signals influencing gene transcription on the molecular level. They are generally found around the transcription start site (TSS) within the gene. It is known that a narrow region around the TSS has distinct nucleotide compositional properties Majewski and Ott [1]. This region, known as the core promoter region, is important in understanding the functionalities of promoters Butler and Kadonaga [2].

The detection of promoter region and the determination of the location of TSS are difficult problems in computational biology. This is mainly due to the fact that the definition of the core promoter region, used by Butler and Kadonaga [2] and Smale and Kadonaga [6], as the minimal continuous segment of DNA sufficient for accurate initiation and direction of transcription, is somewhat incomplete in the sense that it does not specify the length of the...
region. For example, promoter elements TATA box and Initiator always function together to active genetic transcription. However, they have different density distributions in the promoter area. Thus, the study of combinatorial patterns of promoter elements are an important and challenge problem in both biology and computational science.

In this paper we are not only interested in the binding sites that have been identified biologically as those which are initiated by specific transcription factors such as the RNA Polymerase II, but also those short DNA subsequences that have high frequencies in the promoter area even though their biological significance has not been discovered biologically. We call both kinds of subsequences as promoter elements (PEs) in this paper. Regularities in promoter regions are usually studied in terms of occurrence patterns of PEs in them. It is important to note that studies of regularities in promoter regions of homo sapiens reported in the literature thus far, for example, those of Suzuki et al. [8] and Bajic et al. [10], seem to concentrate on the occurrence patterns or over-representation of individual PEs in the promoter regions or their subregions studied. The intention of our study was to investigate pairs of PEs in specific locations with respect to the occurrence or non-occurrence patterns of each member of the pair in relation to the corresponding patterns of the other member. Our study revealed some interesting new relationships that could form the basis for further in-depth analyses of regularities and provide more assistance in the accurate determination of the promoter region and the TSS location.

We studied 20 different promoter elements, some of which have been already identified, and some newly found, in three windows in the promoter area of Homo Sapiens.

For identifying new PEs, we started with an exhaustive search for arbitrary sub-sequences of length of 6~8 nt in the promoter sequences studied and recorded their frequencies of occurrences. Then, we used combinations of several tools for PE finding and PE selection in each of the three windows for our study. This helped us narrow the study to a set of 20 different PEs, each of which might have occurred in one or more of the three windows. By comparing pairs of columns of the binary matrix representing occurrence patterns of the 20 PEs, we were able to identify several interesting regularities that have not been identified thus far.

First, we discovered that when we consider each PE-Window combination as a unique feature, two different features rarely occurred together, and the presence of a select set of features implies the absence of a select of other features. Second, the binary matrix representation also facilitated further analysis of “communities” characterized by richness of a select group of features, called base features, taken one at a time, which revealed more regularities. We defined and calculated a community representation index for each of the other features and discovered that some features are overrepresented while some others are underrepresented in these communities.
MATERIALS AND METHODS

As the promoter data we selected all human promoters which belong to the non-redundant groups as defined in the Eukaryotic Promoter Database (EPD) and the Database of Transcriptional Start Sites (DBTSS). Our data sets included 1,532 genes from EPD and 9,137 genes from DBTSS. We used the region of [-100, +50] relative to the annotated TSS location as given in the two databases. We further subdivided this region into the three overlapping windows $W_1$: [-100, -50], $W_2$: [-60, +10] and $W_3$: [+1, +50], taking into account the fact that the accuracy of the TSS location is within ±10 nt so that any possible imprecision of the annotated TSS can be accommodated.

As non-promoter sequences, we used the coding sequences corresponding to these genes in our data sets [10]. The upstream sequences in the region of [-500, +100] were also used to check positional bias [8]. All those data are downloaded from the European Molecular Biology Laboratory (EMBL) and the National Center for Biotechnology Information (NCBI).

Table 1. In this paper, we focused on 20 different promoter elements (PEs). The list of PEs studied includes those binding sites that have been identified to initiate gene expression and those sub-sequences that have high density and positional bias in promoter area. In the rest of the paper, we will refer to each PE-window pair as a feature characterizing the promoter region. Totally 50 features were studied in our data sets.

<table>
<thead>
<tr>
<th>BIOLOGICALLY IDENTIFIED PEs</th>
<th>NEWLY FOUND PEs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE Names</td>
<td>PE sequences</td>
</tr>
<tr>
<td>TATA</td>
<td>TATAAA</td>
</tr>
<tr>
<td>BRE</td>
<td>SSRCGCC</td>
</tr>
<tr>
<td>Inr</td>
<td>YYANWYY</td>
</tr>
<tr>
<td>Med-1</td>
<td>GCTCCS or SGAGGC</td>
</tr>
<tr>
<td>CAAT</td>
<td>CCAAT or ATTGG</td>
</tr>
<tr>
<td>E</td>
<td>CANNTG</td>
</tr>
<tr>
<td>Sp1</td>
<td>CCGCCC or CMGGTTK</td>
</tr>
<tr>
<td>c-myb</td>
<td>YAACKG or CMGGTTK</td>
</tr>
<tr>
<td>GATA</td>
<td>WGATMR or YKATCW</td>
</tr>
<tr>
<td>PU.1</td>
<td>DVVGAAVY</td>
</tr>
</tbody>
</table>

Among the many promoter elements which have been biologically identified as those that bind to the RNA Polymerase II and initiate gene expression in Homo Sapiens, we selected to study 10 well-known “biologically significant” PEs in each window [7] [8] [9], whose densities in our data sets range from 6% to 30%. However, it should be mentioned that some special PEs, such as DPE, were not considered in this study because we felt that due to their high density of
occurrence, it was not reasonable to study them along with other rarely occurring or studied PEs (see Table 1).

As mentioned earlier, we obtained new PEs by directly enumerating all possible subsequences of length 6–8 nt in the promoter sequences studied and recording their frequencies of occurrences in each window. Simultaneously, we used Gibbs sampling (AlignACE) for the initial PE-finding [12].

To ensure that those subsequences have positional bias in the promoter area, we tabulated the frequencies of occurrences of each subsequence in twelve disjointed segments in the extended region [-500, +100], and selected those subsequences with a cut-off variance of 7 [8]. Furthermore, we narrowed the selection of new PEs by random bootstrap sampling in each window [11] and by comparing the frequencies of occurrence in promoter sequences with the corresponding frequencies in the non-promoter sequences ([10], see Table 2 for more details). Finally, we selected 10 new PEs, and we decided not to study a PE in a specific window if its frequency of occurrence in that window was less than 5% (see Table 1).

Table 2. We narrow the selection of newly found PEs by random bootstrap sampling in each window and by comparing the frequencies of their occurrence in promoter sequences with the corresponding frequencies in the non-promoter sequences. In this table, for each window, we denote the number of promoter sequences in which a PE occurs by \( f \) (referred to as frequency), frequency of the PE in the randomly sampled sequences by \( f_1 \), and the frequency of the PE in the coding sequences by \( f_2 \). The ratios \( R_1 = f / f_1 \) and \( R_2 = f / f_2 \) have been useful in the final selection of the PEs for our study. The PE sequences are represented using four bases A, T, G, C, and eleven degenerate IUPAC Nucleotide Codes [13].

<table>
<thead>
<tr>
<th>Newly Found PEs</th>
<th>Where</th>
<th>Database</th>
<th>( f )</th>
<th>( f_1 )</th>
<th>( R_1 )</th>
<th>( f_2 )</th>
<th>( R_2 )</th>
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<tbody>
<tr>
<td>SATTGGY</td>
<td>W₁</td>
<td>EPD</td>
<td>89</td>
<td>17</td>
<td>5.24</td>
<td>10</td>
<td>8.90</td>
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<tr>
<td></td>
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<td>DBTSS</td>
<td>470</td>
<td>98</td>
<td>4.80</td>
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<td>6.53</td>
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<tr>
<td>GGGSSSGGGGC</td>
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<td>EPD</td>
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<td>1</td>
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<td>411</td>
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<td>12</td>
<td>10.50</td>
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<td></td>
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<td>101</td>
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<td>EPD</td>
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<td>W₃</td>
<td>EPD</td>
<td>108</td>
<td>7</td>
<td>15.43</td>
<td>11</td>
<td>9.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DBTSS</td>
<td>570</td>
<td>45</td>
<td>12.67</td>
<td>78</td>
<td>7.31</td>
</tr>
<tr>
<td>RYGGCGGC</td>
<td>W₃</td>
<td>EPD</td>
<td>105</td>
<td>10</td>
<td>10.50</td>
<td>24</td>
<td>4.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DBTSS</td>
<td>704</td>
<td>68</td>
<td>10.35</td>
<td>141</td>
<td>4.99</td>
</tr>
</tbody>
</table>
ANALYSIS OF FEATURES

Binary Representation of Features

After selecting the PEs we wanted to investigate, we represented their occurrence patterns in the windows as a binary matrix, in which we used 1 (or 0, respectively) to denote the occurrence (or non-occurrence) of a PE in a specific window (denoted as a feature). Our choice of a binary matrix representation for the occurrence patterns was driven by our observation that each feature that occurred more than once appears in up to 15% of the promoter sequences we examined. For instance, the TATA box in window 1 appears at least once in 6 of the 1532 sequences in EPD and 22 of the 9137 sequences in DBTSS, and it never occurs more than once in either database in any of the three windows studied. This behavior was present in the occurrence patterns of all the 50 features we studied (see Table 3 for more details). Therefore we concluded that it suffices to consider the presence or absence of a feature as opposed to how many times a PE occurred in each window. The compilation of data shown in Table 3 also helped us conclude that it is not necessary to study each PE in all the three windows. Thus the occurrence pattern for these features was represented by \( M_{EPD} \), a 1532 \( \times \) 50 binary matrix for the EPD data, and by \( M_{DBTSS} \), a 9137 \( \times \) 50 binary matrix for the DBTSS data. The rows of these matrices correspond to the promoter sequences. Each column of these matrices represents a feature, namely, a unique PE-window combination.

Definitions of Relationships between Features

We compared the occurrence patterns of features by comparing pairs of columns of the binary matrix representing the data. In-depth analysis was performed based on (i) a similarity score, and (ii) the density of occurrence of 1s.

Let \( M \) be an \( n \times m \) matrix, where \( n \) (rows) represents the number of promoter sequences and \( m \) (columns) the number of features.

Definition 1. Let \( i \) and \( j \) denote any two different columns of \( M \). Then we define the similarity index \( s_{ij} \), as the proportion of promoter sequences in which column \( i \) and column \( j \) have identical entries, either both are 0 or both are 1. Equivalently,

\[
S_{ij} = \frac{1}{n} \sum_{k:M_{ik}=M_{jk}} 1
\]

In other words, \( S_{ij} \) denotes the proportion of the promoter sequences data in which column \( i \) and column \( j \) are identical. We interpreted a very high similarity index as an indication that it is sufficient to study the PE-window combination corresponding to either column \( i \) or column \( j \) and not both.

We noted that comparison of any two columns \( i \) and \( j \) in the matrices \( M_{EPD} \) and \( M_{DBTSS} \) corresponds to studying the frequencies of the ordered pairs \((0, 0)\), \((0, 1)\), \((1, 0)\), and \((1, 1)\) in the submatrices induced by these two columns. Since \((0, 0)\) corresponds to the non-occurrence of either of features, we decided to use more meaningful measures of similarity as follows.
Table 3. This table shows the features (PE-window combinations) we studied and the number of promoter sequences in which each of these features occurred at least once ($f$) and the number of sequences in which each feature occurred multiple number of times ($f_m$). We observe that $f_m$ is much smaller (up to 15%) compared to $f$ for each feature.

<table>
<thead>
<tr>
<th>PE Name</th>
<th>Database</th>
<th>$W_1$</th>
<th>$W_2$</th>
<th>$W_3$</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$f$</td>
<td>$f_m$</td>
<td>$f$</td>
</tr>
<tr>
<td>TATA</td>
<td>EPD</td>
<td>6</td>
<td>0</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td>DBTSS</td>
<td>22</td>
<td>0</td>
<td>320</td>
</tr>
<tr>
<td>Ins</td>
<td>EPD</td>
<td>208</td>
<td>18</td>
<td>354</td>
</tr>
<tr>
<td></td>
<td>DBTSS</td>
<td>1514</td>
<td>175</td>
<td>2409</td>
</tr>
<tr>
<td>BRE</td>
<td>EPD</td>
<td>145</td>
<td>4</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>DBTSS</td>
<td>1100</td>
<td>71</td>
<td>1414</td>
</tr>
<tr>
<td>Med-1</td>
<td>EPD</td>
<td>184</td>
<td>21</td>
<td>274</td>
</tr>
<tr>
<td></td>
<td>DBTSS</td>
<td>1420</td>
<td>126</td>
<td>1914</td>
</tr>
<tr>
<td>CAAT</td>
<td>EPD</td>
<td>255</td>
<td>29</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>DBTSS</td>
<td>1382</td>
<td>141</td>
<td>8347</td>
</tr>
<tr>
<td>E</td>
<td>EPD</td>
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<td>256</td>
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<td></td>
<td>DBTSS</td>
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<td>96</td>
<td>1878</td>
</tr>
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<td>EPD</td>
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<td>10</td>
<td>74</td>
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<td>DBTSS</td>
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<td>585</td>
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<td>404</td>
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<td>DBTSS</td>
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<td>647</td>
<td>3067</td>
</tr>
<tr>
<td>PU.1</td>
<td>EPD</td>
<td>104</td>
<td>2</td>
<td>179</td>
</tr>
<tr>
<td></td>
<td>DBTSS</td>
<td>563</td>
<td>19</td>
<td>986</td>
</tr>
<tr>
<td>c-myb</td>
<td>EPD</td>
<td>64</td>
<td>3</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>DBTSS</td>
<td>391</td>
<td>11</td>
<td>728</td>
</tr>
<tr>
<td>SATTGGY</td>
<td>EPD</td>
<td>89</td>
<td>3</td>
<td>49</td>
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<td></td>
<td>DBTSS</td>
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<td>14</td>
<td>265</td>
</tr>
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<td>GGGSSGGGC</td>
<td>EPD</td>
<td>59</td>
<td>3</td>
<td>58</td>
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<td></td>
<td>DBTSS</td>
<td>411</td>
<td>47</td>
<td>502</td>
</tr>
<tr>
<td>SCGGAAG</td>
<td>EPD</td>
<td>60</td>
<td>2</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>DBTSS</td>
<td>285</td>
<td>12</td>
<td>675</td>
</tr>
<tr>
<td>TTCGGG</td>
<td>EPD</td>
<td>47</td>
<td>0</td>
<td>121</td>
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<tr>
<td></td>
<td>DBTSS</td>
<td>293</td>
<td>9</td>
<td>623</td>
</tr>
<tr>
<td>GRSMMGRRGG</td>
<td>EPD</td>
<td>46</td>
<td>1</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>DBTSS</td>
<td>442</td>
<td>38</td>
<td>529</td>
</tr>
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<td>GCGSMKGS</td>
<td>EPD</td>
<td>86</td>
<td>1</td>
<td>123</td>
</tr>
<tr>
<td></td>
<td>DBTSS</td>
<td>537</td>
<td>46</td>
<td>864</td>
</tr>
<tr>
<td>GGGAGGRR</td>
<td>EPD</td>
<td>46</td>
<td>3</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>DBTSS</td>
<td>515</td>
<td>49</td>
<td>560</td>
</tr>
<tr>
<td>RYGCGGC</td>
<td>EPD</td>
<td>33</td>
<td>1</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>DBTSS</td>
<td>241</td>
<td>19</td>
<td>419</td>
</tr>
<tr>
<td>AAGATG</td>
<td>EPD</td>
<td>11</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>DBTSS</td>
<td>56</td>
<td>1</td>
<td>111</td>
</tr>
<tr>
<td>SATGCGG</td>
<td>EPD</td>
<td>11</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>DBTSS</td>
<td>66</td>
<td>1</td>
<td>77</td>
</tr>
</tbody>
</table>
Definition 2. Let \( i \) and \( j \) denote any two different columns of \( M \). Then we define the maximal non-zero comparison index \( MNZC_{ij} \) as the maximum of the frequencies of the ordered pairs \((0, 1), (1, 0), \) and \((1, 1)\) in the submatrix induced by columns \( i \) and \( j \) expressed as a proportion of the total number of ordered pairs \( n \). Equivalently, if we let

\[
p_{ij}^{0,1} = \frac{1}{n} \quad \text{[frequency of \((0, 1)\) in the submatrix induced by columns \( i \) and \( j \)]},
\]
\[
p_{ij}^{1,0} = \frac{1}{n} \quad \text{[frequency of \((1, 0)\) in the submatrix induced by columns \( i \) and \( j \)]},
\]
\[
p_{ij}^{1,1} = \frac{1}{n} \quad \text{[frequency of \((1, 1)\) in the submatrix induced by columns \( i \) and \( j \)]},
\]

then,

\[
MNZC_{ij} = \max(p_{ij}^{0,1}, p_{ij}^{1,0}, p_{ij}^{1,1}).
\]

We also define the direction of the maximal non-zero content (\( DMNC_{ij} \)) induced by column \( i \) and \( j \) as

\[
DMNC_{ij} = \begin{cases} 
1 & \text{if } p_{ij}^{0,1} = MNZC_{ij} \\
2 & \text{if } p_{ij}^{1,0} = MNZC_{ij} \\
3 & \text{if } p_{ij}^{1,1} = MNZC_{ij}
\end{cases}
\]

Note that the direction information from the above definition is significant in that it provides insight into the relationships between pairs of features. In particular, \( DMNC_{ij} = 1 \) corresponds to the absence of feature \( i \) accompanied by the simultaneous presence of feature \( j \), \( DMNC_{ij} = 2 \) corresponds to the presence of feature \( i \) accompanied by the simultaneous absence of feature \( j \), and \( DMNC_{ij} = 3 \) corresponds to the presence of both features.

Definition 3. Let \( i \) and \( j \) denote any two different columns of \( M \). Then the dominant non-zero comparison index \( DNZC_{ij} \) is defined as the maximum of the frequencies of the ordered pairs \((0, 1), (1, 0), \) and \((1, 1)\) in the submatrix induced by columns \( i \) and \( j \) expressed as a proportion of the total number of nonzero ordered pairs \( p_{ij}^{0,1} + p_{ij}^{1,0} + p_{ij}^{1,1} \). Equivalently,

\[
DNZC_{ij} = \frac{\max(p_{ij}^{0,1}, p_{ij}^{1,0}, p_{ij}^{1,1})}{p_{ij}^{0,1} + p_{ij}^{1,0} + p_{ij}^{1,1}}.
\]

Analysis of the Relationships among PE-Window Combinations

Note that the column indexes \( i \) and \( j \) used in the above definitions range from 1 to 50 since there are 50 features in all. Therefore, a final consequence of these definitions is that the study of occurrence patterns in the two databases now has been reduced to analyzing the structure of the three \( 50 \times 50 \) matrices \( MNZC, DMNC, \) and \( DNZC \) for each of the two databases EPD and DBTSS.
from which we obtained our promoter sequences. Interestingly, the structure of each of the matrices for EPD and the structure of each corresponding matrix for DBTSS were identical. We made the following identical observations for both sets of matrices.

First, the entries in $MNZC$ ranged from 0.1 to 0.3. This indicates that there is a very high-percentage of the ordered pair $(0, 0)$ in all pairs of columns compared. This observation is consistent with the fact that both $MEPD$ and $MDBTSS$ from which $MNZC$ were derived were highly sparse, consisting of only about 9% of 1s.

Table 4. When cut-off thresholds of $MNZC_{ij} > 0.2$ and $DNZC_{ij} > 0.7$ are used to describe the existence of a relationship between any two features $i$ and $j$, the structure of the matrix reveals a partitioning of a subset of the feature set. The occurrence of each feature from the left column of this table implies the non-occurrence of each feature from the right column. Recall that $W_1 = [-100, -50]$, $W_2 = [-60, +10]$, and $W_3 = [0, +50]$.

<table>
<thead>
<tr>
<th>PE-Window Set 1</th>
<th>PE-Window Set 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sp1</strong> in $W_1$, $W_2$</td>
<td>c-myb in $W_1$, $W_2$, $W_3$</td>
</tr>
<tr>
<td><strong>Inr</strong> in $W_2$</td>
<td>GATA in $W_1$, $W_2$, $W_3$</td>
</tr>
<tr>
<td></td>
<td>PU.1 in $W_1$, $W_3$</td>
</tr>
<tr>
<td></td>
<td>SATTGGY in $W_1$, $W_2$</td>
</tr>
<tr>
<td></td>
<td>GGGSSSGGGC in $W_1$, $W_2$</td>
</tr>
<tr>
<td></td>
<td>GGGAGGRR in $W_1$, $W_3$</td>
</tr>
<tr>
<td></td>
<td>SCGGAAG in $W_1$, $W_2$, $W_3$</td>
</tr>
<tr>
<td></td>
<td>TTCCGG in $W_1$, $W_2$, $W_3$</td>
</tr>
<tr>
<td></td>
<td>GRGSMGRRG in $W_1$, $W_2$, $W_3$</td>
</tr>
<tr>
<td></td>
<td>GCGSMKGS in $W_1$, $W_2$, $W_3$</td>
</tr>
<tr>
<td></td>
<td>TATA in $W_1$</td>
</tr>
<tr>
<td></td>
<td>CAAT in $W_2$</td>
</tr>
<tr>
<td></td>
<td>RYGGCGGC in $W_2$, $W_3$</td>
</tr>
<tr>
<td></td>
<td>BRE in $W_3$</td>
</tr>
<tr>
<td></td>
<td>CAAT in $W_3$</td>
</tr>
<tr>
<td></td>
<td>Sp1 in $W_3$</td>
</tr>
<tr>
<td></td>
<td>AAGATG in $W_3$</td>
</tr>
<tr>
<td></td>
<td>SATGGCG in $W_4$</td>
</tr>
</tbody>
</table>

Second, the directional information contained in the $DMNC$ matrices contained only 1s and 2s, but no 3s. This indicates that the ordered pairs $(0, 1)$ and $(1, 0)$ were more significant than the pair $(1, 1)$ based on our criteria. In other words, strong similarities between any two features that may be signaled by the occurrence of both features are insignificant; on the other hand, the presence of one feature accompanied by the simultaneous absence of another feature, seems to
be more significant. While the diverse distribution of features may explain the difficulty associated with studying PEs or PE-based features in the promoter area, this observation leads us to believe that the non-occurrences of features may also be able to describe the environment, biological or computational, around the TSS or the core promoter area.

Third, in order to get more information on the occurrence patterns, for each database we constructed a $50 \times 50$ matrix $A$ defined by

$$A_{ij} = \begin{cases} 
1, & \text{if } MNZC_{ij} > 0.2 \text{ and } DNZC_{ij} > 0.7 \\
0, & \text{otherwise.} 
\end{cases}$$

Interestingly, we found that $A$ had the same structure for both databases. In both cases, $A$ had the structure of the adjacency matrix for a complete bipartite graph induced by a subset of the feature set. In addition, it was even more interesting to see that $DMNC_{ij} = 2$ (corresponding to $(1, 0)$ being the dominant relationship) whenever $A_{ij} \neq 0$. This means that if we let $F$ denote the subset of the features (PE-Window combinations) defining the bipartite graph represented in $A$, then $F$ may be partitioned into two subsets $F_1$ and $F_2$ such that the occurrence of a feature in $F_1$ implies the non-occurrence of any feature in $F_2$. Also, no two features both of which belong to $F_i$ and no two features both of which belong to $F_j$ bear such relationship with respect to their occurrences. Table 4 shows the partition of the set of features. It is also important to note that extending our study of two columns using nonzero ordered pairs to three columns using nonzero triples (or more, say $k$, columns using corresponding nonzero $k$-tuples) did not result in any more partitioning of the feature set. The partition as shown in Table 4 is also consistent with the observation that $Sp1$ in $W_1$ and $W_2$, and Inr in $W_1$ have the highest occurrence densities in our data sets.

**Communities Based on Feature Partitioning**

Studies on the promoter region thus far have been based on specific PEs such as the GC-content and TATA box. The GC-content of a sequence, determined as the ratio of the sum of $G$ and $C$ nucleotides over the total number of nucleotides in the sequence ([14] [15]), has been useful to demonstrate that other PEs have strong preference in areas with different GC-contents. This is natural since the promoter elements themselves have different percentages of $G$ and $C$. Therefore, it is not surprising to find that more GC-rich PEs are present in high GC-content (60% or more) areas [10]. Next, although TATA box is one of the most popular PEs that have been identified to bind with the RNA Polymerase II [6], it is not directly useful to describe or predict the TSS or the core promoter region because of its low density of occurrence (less than 8%) in the data sets for homo sapiens that we studied.
Therefore, we propose a new way to partition the data sets into different communities each of which is based on the existence of a distinct feature (PE-Window combination). We have chosen the features shown in the left column of Table 4. We will call these features \((Sp1 \text{ in } W_1, \text{Inr} \text{ in } W_2, \text{Sp1} \text{ in } W_2)\) the base features in the rest of this section. Each community consists of those genes in which one of the base features occurs. The communities corresponding to pairs of base features may overlap, but we do not consider the overlap as significant because the percentage of overlap does not exceed 10%.

We evaluate the preference properties of other features (PE-Window combinations) in these communities using a community representation index (CRI) for each feature. This index is defined as follows:

Table 5. The features with high and low CRIs in the community based on \(Sp1\) in \(W_1\).

<table>
<thead>
<tr>
<th>Features</th>
<th>EPD Database</th>
<th>DBTSS Database</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(f^1)</td>
<td>(f^c)</td>
</tr>
<tr>
<td>GGGSSSGGGC in (W_1)</td>
<td>58</td>
<td>59</td>
</tr>
<tr>
<td>GRGSMGGRRG in (W_1)</td>
<td>28</td>
<td>45</td>
</tr>
<tr>
<td>Med-1 in (W_1)</td>
<td>78</td>
<td>182</td>
</tr>
<tr>
<td>GATA in (W_2)</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td>c-myb in (W_1)</td>
<td>10</td>
<td>64</td>
</tr>
<tr>
<td>Inr in (W_1)</td>
<td>33</td>
<td>197</td>
</tr>
</tbody>
</table>

Table 6. The features with high and low CRIs in the community based on \(\text{Inr}\) in \(W_2\).

<table>
<thead>
<tr>
<th>Features</th>
<th>EPD Database</th>
<th>DBTSS Database</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(f^1)</td>
<td>(f^c)</td>
</tr>
<tr>
<td>(\text{Inr} \text{ in } W_2)</td>
<td>51</td>
<td>151</td>
</tr>
<tr>
<td>c-mby in (W_2)</td>
<td>23</td>
<td>89</td>
</tr>
<tr>
<td>c-mby in (W_3)</td>
<td>23</td>
<td>63</td>
</tr>
<tr>
<td>GGGSSSGGGGC in (W_2)</td>
<td>7</td>
<td>57</td>
</tr>
<tr>
<td>SCGGAAG in (W_2)</td>
<td>14</td>
<td>114</td>
</tr>
<tr>
<td>SCGGAAG in (W_3)</td>
<td>2</td>
<td>24</td>
</tr>
</tbody>
</table>

Table 7. The features with high and low CRIs in the community based on \(Sp1\) in \(W_2\).

<table>
<thead>
<tr>
<th>Features</th>
<th>EPD Database</th>
<th>DBTSS Database</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(f^1)</td>
<td>(f^c)</td>
</tr>
<tr>
<td>GGGSSSGGGGC in (W_2)</td>
<td>52</td>
<td>57</td>
</tr>
<tr>
<td>GRGSMGGRRG in (W_3)</td>
<td>31</td>
<td>54</td>
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<tr>
<td>BRE in (W_1)</td>
<td>31</td>
<td>94</td>
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<tr>
<td>(\text{Inr} \text{ in } W_1)</td>
<td>28</td>
<td>197</td>
</tr>
<tr>
<td>GATA in (W_1)</td>
<td>11</td>
<td>74</td>
</tr>
<tr>
<td>GATA in (W_2)</td>
<td>4</td>
<td>60</td>
</tr>
</tbody>
</table>
Definition 4. The community representation index (CRI) of the i-th feature is defined as

\[ CRI(i) = \frac{f_i^c}{f_i^e} + \frac{f_{\text{base}}}{N} \]

where \( f_i^c \) is the frequency of i-th feature in the specific community, \( f_i^e \) is the frequency of i-th feature in the entire data set; \( f_{\text{base}} \) is the frequency of the base feature in the entire data set; and \( N \) is the size of the entire data set.

Note that the community representation index (CRI) is able to show high or low density of a feature in a specific community when compared to its density in the entire set. If \( CRI(i) = 1 \), it means the density of the feature in the community is the same as its density in the entire set. Thus, a \( CRI(i) > 1 \) will correspond to the i-th feature being overrepresented in the community, while a \( CRI(i) < 1 \) will correspond to underrepresentation.

As can be seen in Table 5, Table 6 and Table 7, GGGSSSGGGC in \( W_1 \) has high CRI in the community based on \( Sp1 \) in \( W_1 \), GGGSSSGGGC in \( W_2 \) has high CRI in the community based on \( Sp1 \) in \( W_2 \), but GGGSSSGGGC in \( W_1 \) has low CRI in the community based on \( Inr \) in \( W_2 \). Also, \( Inr \) in \( W_1 \) has low CRI in the community based on \( Sp1 \) in \( W_1 \), as well as the community based on \( Sp1 \) in \( W_2 \), but \( Inr \) in \( W_1 \) has high CRI in the community based on \( Inr \) in \( W_2 \). Furthermore, GATA in \( W_1 \) and GATA in \( W_2 \) have low CRI in the community based on \( Inr \) in \( W_2 \), SCGGAAG in \( W_2 \) and SCGGAAG in \( W_3 \) have low CRI in the community based on \( Inr \) in \( W_2 \), and c-mby in \( W_2 \) and c-mby in \( W_3 \) have high CRI in the community based on \( Inr \) in \( W_2 \).

DISCUSSION

In this paper, we studied the occurrence patterns of features (combination of promoter elements and their locations) using different definitions of possible relationships among them. Binary matrix representations for the occurrence patterns as well as for comparisons of features facilitated our analyses. We have defined and used nonzero and dominant comparison indexes to drawn conclusions on the occurrence and non-occurrence relationships of pairs of features. Using these measures, we were able to identify a partitioning of a subset of the feature set that helped us conclude that the presence of \( Sp1 \) in \([-100, -50] \), \( Inr \) in \([-60, +10] \), and \( Sp1 \) in \([-60; +10] \) implied the absence of several of the promoter elements we studied in three windows. Also, using these three features as base features, we were able to draw conclusions regarding the extent to which other features are represented in the core promoter region, using a measure which we called the community representation index for each feature. Based on our study and analysis, we strongly believe that some biological or chemical explanations for the patterns we have discovered will emerge. We also believe that the study of several communities coupled with reasonable features has great potential for the discovery of more interesting relationships such as those we found.
References


ACKNOWLEDGMENTS

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EVALUATING MOVEMENTS OF PRONGHORNS IN WIND CAVE NATIONAL PARK, SOUTH DAKOTA

Jaret D. Sievers, Christopher N. Jacques, Jonathan A. Jenks and Daniel E. Roddy
Department of Wildlife and Fisheries Sciences
South Dakota State University
Brookings, SD 57007

ABSTRACT

Pronghorn (*Antilocapra americana*) were reintroduced into Wind Cave National Park (WCNP) in 1914 and thus, have inhabited the park for nearly a century. A decline in the population during the 1990’s raised concern for the continued existence of pronghorn inside the Park. Historically, pronghorn numbers exceeded 300 individuals but were estimated to be less than 50 individuals during this study. Thus, an evaluation of potential factors contributing to the population decline within the Park was initiated. The objective of our study was to determine home range size and seasonal movements of a declining pronghorn population in WCNP. Radio telemetry was used to monitor movements of 8 adult (>1 year at capture) and 19 neonate (< 1 month at capture) pronghorn from 26 January 2002 to 31 May 2004. We collected 407 visual locations on 8 adult females, 177 visual locations on 3 adult males, and 148 visual locations on 19 neonates during our study. Mean daily distance traveled by 8 radiocollared females was 2.6 km in winter and 2.5 km in summer. Ninety-five percent home range contours calculated for 8 radio collared females were 66.6 km$^2$ during winter and 54.5 km$^2$ during summer. Fifty percent core use contours for 8 radio collared females were 7.2 km$^2$ during winter and 7.3 km$^2$ during summer. The longest summer movement of a radiocollared adult female was 11.5 km, and the longest winter movement was 11.0 km. Results indicated that movements of pronghorns likely contributed, in part, to the population decline in WCNP.

Key words

*Antilocapra americana*, home range, movement, pronghorn, South Dakota, Wind Cave National Park

Daily and seasonal movements, as well as patterns of movement, have been described for pronghorns in several western states (Martinka 1966, Hoskinson and Tester 1980, Boccadori and Garrott 2002). Previous studies of pronghorns have described short distance movements as well as long distance migrations. Snow depth, duration of snow cover, and moisture content of vegetation may initiate autumn and spring pronghorn migrations and contribute to determination of winter ranges. Several studies suggested that pronghorns were opportunistic winter migrants and that individuals migrated only as far as necessary to
minimize effects of environmental conditions (Pepper and Quinn 1965, Bruns 1977, Hoskinson and Tester 1980, Barrett 1982). Pronghorns are adapted to moving long distances to locate and use high quality forage (O’Gara and Yoakum 2004), however, some populations may only move short distances (i.e., ≤ 20 km) between seasonal ranges (Cole 1956, Hoskinson and Tester 1980) or remain non-migratory (Boccadori and Garrott 2002).

Home range use by pronghorns also has been well documented throughout western North America (reviewed by O’Gara and Yoakum 2004). Previous reports of home ranges for adult pronghorns have varied widely and included estimates of 0.2 to 2,873 km² (Kitchen 1974, Canon 1993, Clemente et al. 1995, Bates 2000, Hervert et al. 2005). Knowledge of home range use and seasonal movements by pronghorns in regions within the eastern-most extension of sagebrush steppe communities is limited. To our knowledge, relationships among pronghorn seasonal movements, home range use, and the observed population decline have not previously been documented in WCNP. Thus, the objective of our study was to determine home range size and seasonal movements of a declining pronghorn population in WCNP.

STUDY AREA

Wind Cave National Park encompassed an area of 115 km², with an average elevation of 1,257 m above mean sea level and was situated in Custer County, South Dakota, in the southeast region of the Black Hills (Figure 1). Wind Cave National Park was enclosed by a 2.5-m woven-wire fence, with cattle guards present at all road entrances to prevent movement by ungulates out of the Park.

Figure 1. Pronghorn study area in western South Dakota, 2002-2003, was located in Wind Cave National Park (shaded light gray). Thick black lines delineate South Dakota county boundaries.
Wind Cave National Park was characterized by a mosaic of mixed-grass prairie interspersed with a ponderosa pine dominated forest. Plant species occurring in the mixed-grass prairie within WCNP included Kentucky bluegrass (*Poa pratensis*), blue grama, western wheatgrass, western snowberry (*Symphoricarpos occidentalis*), common juniper (*Juniperus communis*), and northern bedstraw (*Galium boreale*). Distribution of silver and big sagebrush was limited in distribution in WCNP. Plant nomenclature followed Larson and Johnson (1999) and Johnson and Larson (1999).

**METHODS**

We captured adult female pronghorns (≥ 1.5 years old) by net-gun deployed from a helicopter (Krausman et al. 1985) in WCNP during January 2002. We restrained, hobbled, blindfolded, and transported captured individuals to predetermined sites for processing. We aged pronghorns based on incisor wear and replacement (Dow and Wright 1962). We ear-tagged, recorded body measurements (chest and neck circumference, right rear foot length), and assessed body condition of each animal. We monitored rectal temperature continuously throughout the processing period as an indicator of physical stress and released individuals if body temperature exceeded 42 °C. We collected blood samples from each pronghorn by venipuncture of the jugular vein for disease and genetic evaluation. We attached radiocollars (Telonics Inc., Mesa, Arizona; Advanced Telemetry Systems, Isanti, Minnesota; 151 MHz) to each captured pronghorn. Radiocollars were equipped with activity and mortality sensors and switched to mortality mode after the transmitter remained still for ≥ 5 hours. Prior to release, we administered a 5-cc intramuscular injection of a broad-spectrum antibiotic (Dual-Gillin, Phoenix Scientific, St. Joseph, Missouri, USA) to pronghorns and removed blindfolds and restraint straps. We recorded total handling time for each pronghorn. We also recorded capture locations for each pronghorn using a Global Positioning System (GPS). Our animal handling methods were approved by the Institutional Animal Care and Use Committee at South Dakota State University (SDSU; Approval number 02-A002).

We located radiocollared adult female pronghorn 2-3 times per week through May 2004 using a hand-held telemetry receiver and directional antenna (Telonics Telemetry Electronics Consultants, Mesa, Arizona; ICOM, Bellevue, Washington). We radiotracked all individuals until they were visually observed; locations of individuals were then assigned Universal Transverse Mercator (UTM Zone 13N, NAD27 Continental United States) coordinates using a hand-held GPS (Garmin International Inc, Olathe, Kansas). Adult males were not radiocollared; however, we opportunistically collected locations when individuals were observed. Adult males were identified by horn characteristics and variation in neckband coloration (Byers 1997).

We entered all pronghorn locations into an Arcview 3.3 Geographic Information System (Environmental Systems Research Institute, Redlands, California); locations were analyzed to determine daily and seasonal movements of pronghorns within WCNP. We calculated seasonal home range polygons using
the adaptive kernel method with CALHOME software (Kie et al. 1996). We used a parametric t-test to test for seasonal differences in movement. We conducted all tests using a statistical significance level of $P<0.05$ unless noted otherwise (Zar 1984).

RESULTS

We collected 407 independent locations from 8 radiocollared adult female pronghorns from 26 January 2002 to 31 May 2004, of which 283 locations were collected during summer 2002-2003 and 124 locations were collected during winter 2002-2004. We documented no differences in 95% ($t=0.921$, $df=14$, $P=0.373$) or 50% ($t=0.051$, $df=14$, $P=0.960$) winter and summer range size during 2002. Ninety-five percent summer and winter home ranges for adult females were 54.5 km$^2$ ($n=8$, SE=8.7) and 66.6 km$^2$ ($n=8$, SE=9.8), respectively (Figure 2). Fifty percent summer and winter core use contours for adult females were 7.3 km$^2$ ($n=8$, SE=1.7) and 7.2 km$^2$ ($n=8$, SE=1.2), respectively (Table 1). Daily distance traveled by adult females between successive locations averaged 2.5 km ($n=8$, SE=0.15) in summer and 2.6 km ($n=8$, SE=0.20) in winter (Table 1). Mean daily distance traveled during winter and summer seasons was similar ($t=0.655$, $df=8$, $P=0.457$) and ranged from 1.8 km in September and October to 3.6 km in December (Figure 3). The longest distance traveled by a radiocollared pronghorn was 11.5 km over a 2-day period in April 2002. The longest distance traveled in a 24-hour period was recorded for 4 females that traveled 9.6 km in November 2002. We observed 2 radiocollared females in Custer State Park near the border with WCNP during the winter of 2003-04; these observations were the only documented occurrences of radiocollared pronghorn outside the boundaries of WCNP during our study.

DISCUSSION

Previous reports indicate that individual home range sizes are variable and result from differences in habitat quality, population and group sizes, land use history, and season (Kitchen and O’Gara 1982). Consequently, home ranges for adult pronghorns have varied widely and include estimates of 0.2 to 2,873

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Table 1. Seasonal movement data for adult pronghorn females in Wind Cave National Park, 2002.
km² (Kitchen 1974, Canon 1993, Clemente et al. 1995, Bates 2000, Hervert et al. 2005). Not surprisingly, sizes of winter (mean=66.6 km²) and summer (mean=54.5 km²) home ranges for pronghorns in WCNP were within the range of what has previously been reported for pronghorns across western North America. However, winter home range size for pronghorns in WCNP was greater than what has previously been reported for several neighboring states. For example, Bayless (1969) noted that average winter home range size for adult female pronghorns in central Montana was 11.5 km² while Hoskinson and Tester (1980) and Firchow (1986) documented average winter home range sizes of ≤ 37.9 km² for adult female pronghorns in Idaho and Colorado, respectively.

Maximum distances traveled by pronghorns during our study (12 km) also were less than previously documented reports in several western states. For example, Martinka (1966) reported migrations by pronghorns of 160 km in Montana while Riddle (1990) and West (1970) documented winter movements in excess of 320 km in response to severe weather conditions in Wyoming and South Dakota, respectively. However, Boccadori and Garrott (2002) studied a non-migratory herd of pronghorns in Yellowstone National Park, Wyoming and suggested that fencing structures likely limited opportunities for individuals to migrate great distances in response to changing environmental conditions.
Constraints placed on pronghorns in WCNP also may have been different
than obstacles encountered by free-ranging populations. Sievers (2004) noted
only 3 of 584 seasonal movements across boundary fences and out of the Park
during 2002-2003. Thus, fencing structures appeared to effectively prohibit
pronghorn movements across fence boundaries and from establishing seasonal
ranges outside the Park. Additionally, lack of snow accumulation during our
study prevented movement of individuals across cattle guards into and out of the
Park, and also contributed to non-migratory behavior by pronghorns.

O’Gara and Yoakum (2004) stated that vegetation influenced seasonal
movements, distribution, and density of pronghorns more than any other en-
vironmental factor because it provided forage and water, as well as hiding cover
for neonates. Several other studies also hypothesized that pronghorn were op-
portunistic migrants and migrated only as far as necessary to minimize effects of
environmental conditions (Pepper and Quinn 1965, Bruns 1977, Barrett 1982).
However, seasonal movements by pronghorns were not observed during our
study. Furthermore, Jacques et al. (2007) suggested that the WCNP pronghorn
population decline was associated with reduced distribution and diversity of op-
timal forage (i.e., habitat quality) and that reduced habitat quality was influenced
by long-term drought conditions throughout western South Dakota. Quality
and distribution of habitats within and adjacent to WCNP likely were important
factors influencing productivity of the WCNP pronghorn population. Further-
more, we hypothesize that constraints placed on pronghorns limited seasonal
movements into and out of the Park, and hence access to high quality seasonal

![Figure 3. Daily movement data for female pronghorn in Wind Cave National Park, 2002.](image)
forage, and contributed to the decline of pronghorns in WCNP. Rigorous measurements of quality and distribution of habitats within and adjacent to WCNP have not previously been quantified. Similarly, effects of inter-specific competition on pronghorn population dynamics have not previously been documented in WCNP. The pronghorn population decline increases the need for better information on quality and distribution of pronghorn habitat within and adjacent to WCNP and effects of inter-specific competition on reduced population growth. Future investigations should further quantify effects of barrier fences on minimizing pronghorn movements and the potential role of deep trenches along the boundary fence in facilitating movement of individuals into and out of the Park. This information would enable managers to better understand factors influencing seasonal movements of pronghorns within WCNP and effects of temporal changes in habitat and density on pronghorn growth and productivity.

ACKNOWLEDGMENTS

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LITERATURE CITED


Abstracts of Senior Research Papers
presented at
The 92nd Annual Meeting
of the
South Dakota Academy of Science
IMPLEMENTING AN ASYNCHRONOUS BIOINFORMATICS WEB APPLICATION

Abby Hurlburt
University of South Dakota
Vermillion, SD 57069

ABSTRACT

In a traditional Web application, a completing a given task may force a user through a page-by-page process to complete data selections and manipulations—this is very time consuming with large data sets. In 2-d gel electrophoresis, a sample produces a set of images with hundreds of identified proteins on an x-y plane, so sharing the data from just one sample would be tedious at best with a traditional Web application. Instead, I used JavaScript programming to make asynchronous requests and display manipulations. This directed me away from page-by-page processes and toward an entire application on just one Web page. By separating the delivery of data from the delivery of the layout and images, I mimicked a desktop application with a Web site. To accomplish this I used AJAX (Asynchronous JavaScript and XML) functionality from the publicly available Prototype code library to request only the necessary data from the server, only at necessary times during application tasks. Prototype and the Scriptaculous library also contain display manipulations and animations, which helped me to insert the requested data onto the page so that I didn't have to load an entire page for each stage of any given task—just the data itself. This AJAX approach, as opposed to the traditional development of a Web application, improved usability and efficiency by reducing or eliminating waiting time between tasks while data and files are downloaded and uploaded.
YEAST TWO-HYBRID ANALYSIS OF BINDING INTERACTIONS AMONG THE ENTEROHEMORRHAGIC \textit{ESCHERICHIA COLI} EFFECTOR PROTEIN NLEF AND THE MAMMALIAN PROTEOME

Ashley Grajczyk  
Department of Biology and Microbiology  

Eric Brown  
Veterinary Science Department  
Center for Infectious Disease Research and Vaccinology  

Philip R. Hardwidge  
Department of Biology and Microbiology  
Veterinary Science Department  
Center for Infectious Disease Research and Vaccinology  

South Dakota State University  
Brookings, SD 57007

ABSTRACT

Pathogenic strains of \textit{Escherichia coli} are responsible for many diseases, including meningitis, urinary tract infections, and diarrhea. These bacteria attach to the intestinal wall and create attaching/effacing lesions, but little is known about \textit{E. coli} effector proteins and how they specifically cause diarrhea. We hypothesized that an effector protein named NleF is an essential virulence protein that associates with intestinal epithelial proteins involved in intracellular protein trafficking and protein secretion. To test our hypothesis, we utilized a yeast two-hybrid assay to discover human proteins that interact with NleF. A beta-galactosidase assay was employed to validate findings from the two-hybrid screen. We identified six different human cDNAs sequences that may encode proteins that interact with NleF. These interactions will be confirmed by coimmunoprecipitation and coimmunolocalization in infected intestinal cells. Elucidation of the molecular mechanism by which NleF subverts host cell defenses has significant implications for treatment and prevention of diarrheal disease.
VEGETATION ESTABLISHMENT AND SHORT-TERM SUCCESSION ON THE RUBY GULCH WASTE ROCK REPOSITORY IN THE NORTHERN BLACK HILLS OF SOUTH DAKOTA

A. C. Korth and G. E. Larson
Department of Biology/Microbiology
South Dakota State University
Brookings, SD 57007

ABSTRACT

The Ruby Gulch Waste Rock Repository was created in 2001 to sequester heavy metal- and acid-generating rock exposed by past gold mining at the Gilt Edge Mine site in the northern Black Hills. Repository construction included the placement of a polyethylene membrane over waste rock deposited in Ruby Gulch, a tributary of Strawberry Creek, and burying it beneath approximately 150 cm of rock and soil materials. The surface of the waste rock cap, much of it characterized by erodible 30 percent slopes, was seeded with a grass-forb mixture in 2003. Our purpose was to assess successional trends and long term sustainability of vegetation established on the cap. Fifty-six 1-m² plots, and 20, 20-m transects were permanently established in 2005 to annually measure canopy cover, ground surface cover, and species diversity. Data collection included ocular estimation of canopy cover for the 1-m² quadrats and for 0.25-m² microplots regularly positioned along the transects (10 microplots per transect). A point frame was used to measure ground cover along each transect. Data from 2005 and 2006 indicate a decrease in cover and species richness, including a decline in broad-leaved plants (especially clover, *Trifolium* spp.), a disappearance of western wheatgrass (*Pascopyrum smithii*), and thickspike wheatgrass (*Elytrigia dasystachya*), and little vegetative cover at the soil surface. Intermediate wheatgrass (*Elytrigia intermedia*), slender wheatgrass (*Elymus trachycaulus*), and hard and sheep fescues (*Festuca* spp.) appeared most constant between years. Severe drought and a grasshopper outbreak in 2006 contributed to vegetation changes observed between 2005 and 2006. Continued monitoring should determine whether these changes are permanent or temporary.
Nesting success of mallards (*Anas platyrhynchos*) and ring-neck pheasants (*Phasianus colchicus*) is widely studied. However, no previous studies focusing on this have been conducted at Oak Lake. A baseline assessment was needed to assess causes of nest mortality and to determine overall success. It was anticipated that mammalian predators would be a major cause of nest failure and that overall nesting success would be low. Non-random searches with a hockey stick and a circular drag line method were conducted from May 15, 2006 to June 26, 2006. Nests were determined to be successful if one egg hatched. Eggs were said to have hatched if detached shell membranes were found with shell fragments. Vegetation visual obstruction was assessed using a Robel pole at each nest. Five nests were monitored, three mallard and two ring-neck pheasant. Mammalian predators predated two mallard nests and one pheasant nest. Controlled burning destroyed one mallard nest. One pheasant nest was abandoned. None of the five nests were successful. There was no significant difference with 95% confidence between mallard and ring-neck pheasant nest site selection in terms of vegetative visual obstruction ($p = 0.053$).
PHASE TRANSITIONS IN LARGE RARE GAS CLUSTERS

David T. Huebner and Brian G. Moore
Chemistry Department
Augustana College
Sioux Falls, SD, 57197

ABSTRACT

The phase behavior of clusters of atoms interacting through a dispersive (Lennard-Jones) potential was simulated using constant energy molecular dynamics and constant temperature Monte Carlo methods. The liquid cluster phase has never been systematically followed to larger sizes. Previous work has suggested that at some point sublimation intervenes and the liquid state is not stable. To follow liquid clusters to larger and larger sizes, we have been developing methods for reducing computation time and new ways to analyze the cluster as it evolves. Neighbor lists, switching functions, and parallel computing in both dedicated rack mounted and non-dedicated computer lab environments were explored. In addition, the functionality of the molecular dynamics code was expanded from Lennard-Jones systems to also allow for the simulation of ionic systems.
A DNA BAR-CODING APPROACH TO ASSESS THE BIODIVERSITY OF BLACK HILLS ARACHNIDS

Emily J. Chiller, Cynthia Anderson and Shane K. Sarver
Center for the Conservation of Biological Resources
Black Hills State University
Spearfish, SD 57799

ABSTRACT

The purpose of this study is to collect data on Arachnid (infraorder araneomorphae) populations in the Black Hills of South Dakota, which has not previously been studied. This lack of information is a serious impediment to any detailed studies in this field. Collecting arachnid specimens and genetic data for use in taxonomic identification creates a foundation for research into arachnid species distribution, genetics, and ecology. We have been able to preserve morphological integrity of specimens while extracting DNA, enabling the preservation of both molecular and morphological characteristics for taxonomic identification. Ideally, this method of DNA extraction will provide a means to collect genetic sequence data from holotypes while preserving the integrity of the specimen in future studies. The creation of a database of genetic bar codes for arachnids will be of great value to the arachnological community, as will the data regarding species occurrences in the Black Hills.
NEWLY SYNTHESIZED BICYCLIC QUINONES AS POTENTIAL ANTI-OVARIAN CANCER AGENTS

Christine A. Magee, Alicia A. Goyeneche, Grigoriy A. Sereda and Carlos M. Telleria
Division of Basic Biomedical Sciences and Department of Chemistry
University of South Dakota
Vermillion, SD 57069

ABSTRACT

Doxorubicin, which is used to treat ovarian cancers that develop resistance to standard carboplatin-paclitaxel chemotherapy, was first shown to have antitumor activity in the 1960’s. Doxorubicin, however, is cardiotoxic. Three approaches are being followed in our laboratories to improve doxorubicin efficacy: 1) combining doxorubicin with the chemosensitizing agent Mifepristone (RU486) that may allow doxorubicin to achieve similar cytotoxicity with less dose; 2) synthesizing new diquinones with anti-cancer properties; and 3) generating quinones-porphyrin conjugates for photodynamic therapy. Experiments involved treating ovarian cancer cells with doxorubicin, with quinone compounds intended to be attached to a porphyrin moiety, and with a combination of RU486 and doxorubicin. Doxorubicin was found to be highly cytotoxic to OV2008 ovarian cancer cells. Quinone compounds #4-7 were found to be good candidates for linkage to a porphyrin moiety. In OV2008 ovarian cancer cells, pretreatment with RU486 followed by doxorubicin exposure is more effective at causing cell death than RU486 alone, doxorubicin alone, or co-treatment of RU486 + doxorubicin.
PRELIMINARY STUDY FOR GIARDIASIS DRUG DEVELOPMENT

Brandi Tredeau, Bethany Bleich, Cindy Konopasek and Chun Wu
Division of Natural Sciences
Mount Marty College
Yankton, SD 57078

ABSTRACT

Giadiasis is the most common form of non-bacterial diarrhea in North America. It is caused by the parasite Giardia lamblia. Giardia lamblia is classified as a category B organism in response to bioterrorism threats by the Centers for Disease Control and there are over 2.5 million cases of giardiasis occur annually in the United States. Currently there is no FDA approved medicine available. The long-term goal of this project is to develop new drug candidates for alternative treatments of Giardiasis.

Class II Giardia fructose-1,6-diphosphate aldolase catalyzes the reversible condensation of dihydroxyacetone phosphate with glyceraldehyde 3-phosphate to produce D-fructose 1,6-bisphosphate in glycolosis, a central metabolic pathway. Class II Giardia fructose-1, 6-diphosphate has been shown to be essential to Giardia lamblia growth by RNAi gene knock-out experiment. In addition, this enzyme does not exist in human cells. Therefore, it is an ideal anti-parasitic drug target.

This poster reported on the rational design of Giardia aldolase inhibitors. A novel active site filling model was applied. It included molecular modeling via a Linux workstation (e.g. Insight II, Autodock 3.0 and Ludi). The synthesis of major intermediates of those candidates was reported.

The future work will include in-vitro inhibitor evaluation (e.g. layout of bioassays, Michaelis-Menten kinetics, Lineweaver-Burk plots and enzyme inhibition, etc.). Once a nanomolar level inhibitor with high specificity is identified, development of the X-ray crystal structure of enzyme inhibitor complex will be performed followed by in-vivo evaluation.
DEVELOPMENT OF A CAPTIVE REARING PROGRAM FOR THE ENDANGERED HINE’S EMERALD DRAGONFLY (SOMATOCHLORA HINEANA)

C. D. Satyshur and D. A. Soluk
Biology Department
University of South Dakota
Vermillion, SD 57069

ABSTRACT

The Hine’s emerald dragonfly (Somatochlora hineana) is a federally-listed endangered species occurring in isolated locations in the mid-western US. Larvae take 3-5 years to emerge and require a habitat that features clean, spring and seep fed streamlets that dry for at least part of the year. Their life cycle is unusual in many ways including the fact that larvae of this species spend much of the year in the burrows of the predatory devil crayfish (Cambarus diogenes). This behavior allows the larvae to escape streamlet drying and winter freezing. Continuing threats from habitat destruction and climate change prompted development of captive rearing protocols beginning in 2003. Successful methods for rearing larvae larger than instar F-7 were standardized in 2006. Larvae were housed individually in containers with uniform habitat structures and water from habitat areas. Temperature and feeding regimes mimicked those in Door County, WI, which currently supports the largest population of Hine’s. During summer months, most larvae have been placed in the Door County habitat in cages that allow natural food sources, temperatures and light regime while protecting larvae from predators. Head width, total length and weights of captive larvae were similar to those of wild caught larvae, indicating that captive growth rate is appropriate. In 2006 the successful emergence rate was 94.3% and overall mortality for captive larvae was under 7%. The development of successful rearing protocols is a critical step in conserving and reintroducing the Hine’s emerald dragonfly into areas from which it has been extirpated.
SPINY SOFTSHELL, SMOOTH SOFTSHELL AND FALSE MAP TURTLE NEST SITE HABITAT CHARACTERISTICS ALONG THE MISSOURI NATIONAL RECREATION RIVER IN SOUTH DAKOTA

Laura A. Dixon and Charles D. Dieter
Department of Biology and Microbiology
South Dakota State University
Brookings, SD 57007

ABSTRACT

Little is known about the ecology and reproductive habits of turtles in South Dakota. The spiny softshell (Apalone spinifera) and smooth softshell (A. mutica) are listed as species of concern in South Dakota and the false map turtle (Graptemys pseudographica) is listed as state threatened. Information relating to habitat and nest site characteristics is needed to form sounds management plans. Surveys were conducted for turtle nests along the Missouri National Recreation River from Gavin’s Point Dam to Ponca State Park beginning in May through August of 2006. Turtle nests were located by walking shorelines and sandbars while searching for predated nests, tracks, scrapes and nesting turtles. Once located, nests were identified to species and recorded on GPS. Nest and habitat characteristics such as number of eggs, egg size, depth and width of nest, surface temperature, soil temperature, substrate, distance from water, distance to nearest vegetation and type, and estimated percent sunlight exposure were also recorded.

Turtles were first observed nesting on June 5th and nesting continued until July 23rd 2006. A total of 4 false map and 16 softshell nest were located intact and excavated for measurements. One false map and 9 softshell predated nests were also located and recorded. Field work will continue in 2007.

Keywords

Apalone spinifera, Apalone mutica, Graptemys pseudographica, nesting habitat, Missouri River
KINETICS OF TRIOCTYLMETHYLAMMONIUM METHYL CARBONATE

Chris Fry, Kara Becvar, Gary W. Earl and Duane E. Weisshaar
Chemistry Department
Augustana College
Sioux Falls, SD 57197

ABSTRACT

Quaternary ammonium compounds (quats) are widely used for a variety of commercial products including fabric softeners, hair conditioners, and cleaners. Industrially these compounds are produced using hazardous reagents like dimethyl sulfate and methyl chloride. We have developed an eco-friendly route for the methylation of tertiary amines to form the quat using dimethyl carbonate (DMC). Previous studies in this lab determined rate constants and energies of activation for the reactions of tributylamine, trihexylamine, and trioctylamine with DMC at three temperatures, using ion exchange HPLC with an acidic eluent to analyze the reaction mixture. Unexpected trends coupled with poor chromatographic resolution for the trioctylamine and corresponding quat led us to repeat that segment of the work. After adding data from three additional temperatures, the new results were consistent with the previous work. Rate constants increased with increasing temperature. The Arrhenius plot yielded an energy of activation of +77 kJ/mole. The composite results for the trioctylamine system clearly indicated data from more than three temperatures are needed to adequately define the Arrhenius plot.

INVESTIGATING THE REACTION OF DIPHENYLAMINE WITH DIMETHYL CARBONATE

Melissa St. Aubin, Paul Draayer, Alli Maenke, Gary W. Earl and Duane E. Weisshaar
Chemistry Department
Augustana College
Sioux Falls, SD 57197

ABSTRACT

Quaternary ammonium compounds (quats) are used commercially for a variety of applications. They are produced industrially by methylating tertiary amines with relatively hazardous methyl chloride or dimethyl sulfate. We have developed a green route for the methylation using dimethyl carbonate (DMC). With a 10:1 excess of DMC at 135°C (high pressure reactor required) the reaction with aliphatic amines was complete in 7-12 hours. Excess DMC and methanol solvent were successfully recovered and recycled.

To test the process with aromatic amines and extend the scope of the methylation to secondary amines, the double methylation of diphenylamine was attempted. Reaction at 135°C with a 20:1 excess of DMC produced no products after 48 hours. Adding K₂CO₃ to remove the proton after the first methylation under the same conditions also produced no reaction. Increasing the temperature to 180°C resulted in formation of a carbamate instead of the quat. Tundo et al. indicate that with DMC carbamate formation occurs at lower temperatures than methylation, so perhaps a higher temperature is needed to form the dimethyldiphenylammonium methyl carbonate compound.

TRADING PLACES: ANION EXCHANGE OF QUATERNARY AMMONIUM COMPOUNDS

Paul Draayer, Alli Maenke, Missi St. Aubin,
Gary W. Earl and Duane E. Weisshaar
Chemistry Department
Augustana College
Sioux Falls, SD 57197

ABSTRACT

Quaternary ammonium compounds (quats) are used commercially for a variety of applications, many of which are dependent on the counter anion. We have developed a green route for the methylation of tertiary amines to form the quaternary using dimethyl carbonate (DMC). This work explored methods for exchanging the methyl carbonate anion that resulted from the reaction with DMC. The first attempt via a direct exchange with methoxide or hydroxide (strong base) by precipitation of potassium methyl carbonate in methanol solvent produced poor yields due to solubility of the potassium methyl carbonate in methanol. Other solvents proved to be equally problematic. The next attempt using Dowex 1-X8 anion exchange resin worked well for methoxide, lactate, and benzoate. This multi-step process required conversion of the exchange resin to the appropriate form by reaction with an appropriate salt, rinsing the resin, and finally reacting with the methyl carbonate quaternary. The third attempt via direct reaction of the methyl carbonate with an appropriate acid using a vacuum to drive the reaction to completion also worked well. Direct reaction is the most straightforward method for reaction with acids stronger than hydrogen methyl carbonate. The anion exchange method is required for substituting anions of acids weaker than hydrogen methyl carbonate.

INFLUENZA A MATRIX PROTEIN IS NECESSARY BUT NOT SUFFICIENT FOR FORMATION OF VIRUS-LIKE PARTICLES

A. H. Harmon, Y. Weng, D. Wan, E. Nelson, and F. Li
Department of Veterinary Science
South Dakota State University
Brookings, SD 57007

ABSTRACT

The final stage of the influenza replication cycle requires the assembled virus to envelope itself and bud from the plasma membrane (PM) of infected cells. This process is likely mediated through interactions of the assembly domains encoded on the Matrix protein (M1) and host proteins. Based upon this, it has been concluded that the M1 protein alone can bud and form virus like particles (VLP). This has been supported by two studies using either Cos-1 cells (fibroblast type cell) or an insect SF9 cell line. Only epithelial cells can become infected with Influenza, so we sought out to determine if VLPs can be formed with M1 protein alone in epithelial cells (MDCK line). Upon transfection, we were unable to detect any VLP production with a viral budding assay, though cellular accumulation of M1 protein was detected. When both fluorescently labeled M1 and WT M1 proteins were evaluated by immunostaining, the M1 protein appeared to be localized in or near the nucleus (confirmed by nuclear staining), not by the PM, which is necessary for viral assembly and budding. The M1 protein contains a nuclear localization signal which is responsible for initial trafficking of the protein into the nucleus, so our results were not unexpected. Once in the nucleus, M1 must bind viral RNA nucleoproteins (vRNPs) and the nuclear export protein (NEP) to be exported out of nucleus and begin trafficking towards the PM. This work is the initial effort to a comprehensive study of M1 trafficking.
EFFECTS OF LEAVES OF DIFFERENT COLOR ON GROWTH OF SELECTED BACTERIA

Casey Brown and Donna Hazelwood
College of Arts and Sciences
Dakota State University
Madison, SD 57042

ABSTRACT

Recent studies in our laboratory have indicated that differently colored leaves have an effect on the growth of selected bacteria. Samples of leaves from trees and shrubs were collected before and after leaves changed color, dried, ground, and measured amounts were added to nutrient agar before autoclaving. Bacterial suspensions were then plated on agar amended with leaves. Nutrient agar plates served as control. Bacteria-leaf combinations were recorded as positive if visible growth was observed. If growth was not visible, transfers were made to nutrient broth and observed for subsequent growth. Growth was observed on all bacteria nutrient agar combinations. For *E. coli* B, visible growth was recorded for each of the leaf amended nutrient agars except for both the red and the green oak leaves. With the exception of green and yellow walnut leaves, growth was recorded for *Citrobacter freundii* on all other leaf-agar combinations. For *Micrococcus luteus*, growth was present only on linden samples. Finally, growth was not observed on any of the leaf amended agars for *Micrococcus roseus*. Follow up testing from plates with no visible growth was performed by inoculating nutrient broth, and revealed that growth was observed for *E. coli* on green oak. In contrast, growth was not observed on any of the other samples tested in broth.

Conversely, growth was not observed for certain bacteria-leaf combinations. Allelopathic effects appear to be specific for the bacteria and plant species and leaf color combinations. Further studies will continue examination of these phenomena.
FIELD VALIDATION OF A MULTIPLEX PCR ASSAY AND QUANTITATIVE MULTIPLEX PCR ASSAY FOR DIFFERENTIATING AND QUANTIFYING RUMINANT TRICHOSTRONGYLE EGGS FROM SOUTH DAKOTA CATTLE

Zachary B. Williams
Department of Biology & Microbiology
South Dakota State University
Brookings, SD 57007

A.F. Harmon
Center for Infectious Disease Research & Vaccinology
Department of Veterinary Science
South Dakota State University
Brookings, SD 57007

Dante S. Zarlenga
USDA, ARS, Bovine Functional Genomics Lab
Beltsville, MD

Tom A. Yazwinski
Department of Animal Science
University of Arkansas
Fayetteville, AR

Michael B. Hildreth
Department of Biology & Microbiology
Center for Infectious Disease Research & Vaccinology
Department of Veterinary Science
South Dakota State University
Brookings, SD 57007

ABSTRACT

The purpose of study was to use production herd fecal samples to evaluate a traditional multiplex assay (MPCR) and real-time multiplex PCR assay (QPCR) for identifying cattle trichostrongyle eggs to the genus level in these samples, and also for estimating the relative number of eggs from each genus (QPCR only). Fecal samples from cattle herds in eastern South Dakota were used to determine specificity and sensitivity of these assays as well as quantitative capacity for the QPCR assay. Eggs were isolated and counted using a modified Wisconsin protocol; they were disrupted with a bead beater. A DNeasy Plant kit (Qiagen) was used to isolate DNA. Larval culture and identification was also performed on
samples from 4 herds and compared with results from both assays. MPCR and QPCR was carried out using primers, probes and conditions designed for *Hae-
monchus, Ostertagia, Trichostrongylus, Cooperia* and *Oesophagostomum* (MPCR only). Agreement reached 93% between both assays and larval identification, while both assays agreed with each other in 100% of the adjusted samples. When archived samples from 13 herds were amplified, MPCR resulted in sensitivity of 79% and a specificity of 79%; QPCR resulted in a sensitivity of 87% and specificity of 70%. Two similar studies with human hookworm eggs resulted in sensitivities which were similar to our findings. This use of naturally infected herds from South Dakota was not effective for properly evaluating the quantitative capacity of these assays; however, these results indicate that both PCR assays should be sufficient if not more accurate than the current labor intensive larval identification process.
EFFECTS OF TEMPERATURE FLUCTUATIONS ON ADULT *CULEX TARSALIS* COLLECTIONS DURING 2005 IN EASTERN SOUTH DAKOTA

Mitch McKenzie, Matthew J. Wittry, Ryan Beyer, Clayton Wulf, Jake Schaeffer and Michael B. Hildreth

Departments of Biology/Microbiology and Veterinary Science
South Dakota State University
Brookings, SD 57007

ABSTRACT

*Culex tarsalis* is the primary vector for West Nile Virus throughout the western USA, and municipalities in this region are using the number of *Cx. tarsalis* adults collected in CO2–baited light traps for assessing the need for adulticide control measures. Typically, 1-3 collections are made each week, with the assumption that they are representative of the existing population. During the past 5 years, daily collections were made at an acreage near Brookings, SD which documented the yearly, seasonal expansion of the *Cx. tarsalis* population. A CDC miniature light trap baited with CO2 regulated by a photocell-controller was used each year to make the collections. Though the general population expansion trend was consistent each year, there was considerable daily variation within the trend. The purpose of the present study was to determine if daily temperature fluctuations correlated with fluctuations in daily *Cx. tarsalis* collections. For this study, data collected during 2005 was used because the population of *Cx. tarsalis* expanded greatly during that summer. From the temperature data, daily highs and lows that were at least one standard deviation from the average daily temperature were selected and compared to the fluctuations seen in *Cx. tarsalis* numbers collected during that day, and one and two days later. Using this approach, no correlations were observed. Because barometric pressures and wind velocity/direction also regularly fluctuate on a daily basis, these weather conditions should also be evaluated for correlations with the collected *Cx. tarsalis* numbers.
WEST NILE VIRUS INFECTION RATES OF CULEX TARSALIS AND THE RATIOS OF Aedes vexans TO Culex tarsalis DURING MULTIPLE YEARS AT MULTIPLE TRAPPING SITES IN SOUTH DAKOTA

Colin E. Brown, Matt J. Wittry and Michael B. Hildreth
Departments of Biology/Microbiology and Veterinary Science
South Dakota State University
Brookings, SD 57007

Rachel A. Hoffman and Christopher D. Carlson
South Dakota Public Health Laboratory
Pierre, SD

ABSTRACT

Since 2003, West Nile Virus (WNV) has been a major health concern for South Dakotans. The main vector for WNV in the state is Culex tarsalis. This vector mosquito and the nuisance mosquito, Aedes vexans, are the major two mosquito species feeding on humans throughout this region. Aedes vexans rarely transmits WNV, but moderate populations of this nuisance species elicit avoidance behaviors in people (e.g. going indoors, wearing mosquito repellants) that might also limit the exposure of these individuals to WNV from the vector mosquito. This study compared Ae. vexans and Cx. tarsalis populations in three ecogeographic regions of South Dakota (Brookings, Huron, and Pierre) over a four-year period (2003 to 2006). Mosquitoes were collected using CDC miniature light traps baited with carbon dioxide so that they could also be tested for WNV using an RNA III Isolation Kit and RT-PCR procedures. During the four-year span, 92.5% (245 out of 265) of the positive pools throughout the state were observed during a transmission period which ran from July 8th to August 31st. During the pre-transmission period (June 1-July 7), there was great variability in mosquito numbers each year and site (ranging from 958.9 Ae. vexans /day during the wet year of 2004 in Brookings to 2.8/day during the drought year of 2006 in Brookings); in most cases, the ratio of Ae. vexans to Cx. tarsalis mosquitoes was greater than 5:1. During the transmission period, the mean number of mosquitoes/day generally ranged between 100 and 400, and there was an increased percentage Cx. tarsalis. The Huron and Pierre sites generally had a higher percentage of Cx. tarsalis than Brookings.
PRODUCTION OF VOLATILE FATTY ACIDS
USING WHOLE STILLAGE

Emily Snyder and William Gibbons
Center for Bioprocessing
Department of Biology and Microbiology
South Dakota State University
Brookings, SD 57007

ABSTRACT

The objective of this study is to convert whole stillage (a byproduct of corn ethanol production), into a mixture of volatile fatty acids (VFAs). These VFAs will subsequently be converted to polyhydroxyalkanoate via Ralstonia eutropha. We have acclimated a mixed rumen culture to produce more VFAs and less gas when grown on whole stillage. Acclimation was carried out by repeatedly subculturing the rumen consortium on whole stillage that contained low levels of ionophores (Rumensin, Bovatec, and Cattylst) which repressed gas producing microbes. After several such transfers, the rumen culture maintained low production of gas even when the ionophores were no longer added. Unfortunately, this acclimation also resulted in the reduction or loss of cellulase producing microbes, which can also be inhibited by ionophores. To increase cellulose digestion and VFA production, we will evaluate various pretreatments and/or addition of commercial cellulase enzymes or microbes that produce cellulase. Initial trials evaluated a group of six commercial hydrolytic enzymes, obtained from Novozyme, at recommended and 10X recommended rates. Saccharification trials were conducted at 50° Celsius for 72 hours, with routine HPLC analysis. Results indicated that the enzyme, NS50012, at the recommended rate was the best for increasing VFA production based on sugar concentration after 72 hours. Subsequent trials will evaluate combinations of the best enzymes at the recommended rate. Pretreatments to be evaluated will include hot cook and near critical water treatments, in which temperature is the primary variable. We also plan to explore the feasibility of re-introducing cellulase producers into the acclimated rumen consortium.
PLANT HEIGHT, COLEOPTILE LENGTH, AND GIBBERELLIC ACID SENSITIVITY OF U.S. GREAT PLAINS WINTER WHEAT GERMLASM

Frederic Hakizimana and Amir M. H. Ibrahim
Plant Science Department
South Dakota State University
Brookings, SD 57007

ABSTRACT

In the northern Great Plains of the US, optimum fall establishment is critical for winter survival of winter wheat (Triticum aestivum L.). The coleoptile length (protective sheath that covers the shoot during emergence) has been associated with fall stand establishment, most notably with semi-dwarf wheat cultivars that possess the Rht1 and/or Rht2 semi-dwarfing genes. Little information is known on Great Plains winter wheat coleoptile length. The objectives of this study were 1) to identify sources of adapted semi-dwarf winter wheat germplasm with long coleoptile development, and 2) to obtain information on coleoptile length of the standard height winter wheat genotypes. Seeds of 143 winter wheat genotypes from the Uniform Winter-hardness Nursery (UWHN)-Southern Great Plains section (S) and 131 from the Northern Great Plains section (N) were evaluated. Forty seeds of each genotype were germinated and the standard normal deviates (Z-values) showed that eighteen genotypes with Rht1 and/or Rht2 semi-dwarfing genes from the UWHN-S and eight from the UWHN-N had long coleoptiles while four genotypes with Rht8 or Rht9 from the UWHN-N had long coleoptiles. These results indicated that semi-dwarf genotypes with long coleoptiles exist among the southern and northern Great Plains winter wheat. These genotypes will be useful to wheat breeders interested in incorporating Rht dwarfing gene(s) into their germplasm while maintaining coleoptile length.
EVALUATION OF SOUTH DAKOTA WINTER WHEAT FOR FUSARIUM HEAD BLIGHT RESISTANCE

Subas Malla and Amir M.H. Ibrahim
Plant Science Department
South Dakota State University
Brookings, SD 57007

ABSTRACT

Fusarium head blight (FHB) is an important wheat disease in the U.S. We investigated genetic diversity of FHB resistance in advanced winter wheat. A total of 84 hard winter wheat genotypes representing the Advanced Yield Trial (AYT) and Crop Performance Testing (CPT) were artificially inoculated in a mist-irrigated field in 2003 and 2004. Forty-four genotypes, including 22 from the CPT, were also evaluated in a greenhouse in 2003. Genotypes varied significantly ($P < 0.01$) for flowering date, disease index (DI) and percent Fusarium damaged kernel (FDK) in both years. No correlation was found between DI and FDK, except in the AYT in 2003. DI and FDK varied significantly ($P < 0.01$) in the CPT in both years and showed significant genotype-by-year interaction ($P < 0.05$), which emphasizes the importance of multi environment and year screening. Genotypes also varied significantly ($P < 0.01$) for DI in the greenhouse. No correlation was observed for DI between the greenhouse and the field. DON content ranged from 17.0 ppm to 56.0 ppm in 2004, indicating lack of resistance to DON accumulation in these genotypes. Nivalenol accumulation was low (< 0.05 ppm) in all genotypes. DON content was correlated ($r = 0.6$ and $0.5$, $P < 0.05$) with both FDK and DI, respectively. This indicates that the DI and FDK should be recorded separately to assess FHB resistance.
A SECONDARY FERMENTATION OF DISTILLERS GRAINS BY SCLEROTIUM GLUCANICUM WILL ADD VALUE TO ETHANOL PRODUCTION BYPRODUCTS

James Ekenstedt, William Gibbons and Jeremy Javers
South Dakota State University
Brookings, SD 57007

ABSTRACT

Adding value to ethanol production byproducts is an interest of the industry. Currently, the common process is to dry distillers’ grains with solubles (DDGS) to a low-moisture, granular product that is typically fed in a confined feeding operation. Storage and handling of this product can be problematic due to crusting and bridging that can occur in bins, railcars, and trucks. One way to avoid these handling problems would be to pelletize DDGS. This would also permit open-range feeding of livestock, thereby opening additional markets. However, pelletizing would require that an adhesive be added to the DDGS. We have investigated several gum producing organisms to use in a secondary fermentation that would confer adhesive properties to DDGS. These microbes, Sphingomonas paucimobilis, Sclerotium glucanicum and Xanthomonas campestris were first acclimated to grow on whole stillage by incrementally increasing the amount of stillage (or stillage component) in the hybrid medium, while decreasing the level of the defined medium used. Lab scale (4 L) fermentations will be performed to identify the optimal medium formulation (including nitrogen supplementation rate), along with physical parameters such as agitation, aeration, and pH, in order to maximize gum production.
TILLAGE EROSION COEFFICIENT MEASUREMENTS

J.Q. Mollinedo, J.A. Schumacher, T.E. Schumacher
Plant Science Department
South Dakota State University
Brookings, SD 57007

S.Li and D.A. Lobb
Soil Science Department
University of Manitoba
Winnipeg, MB, R3T 2N2

ABSTRACT

Soil loss as a result of differential translocation of soil by tillage is widespread in regions with irregular topography. Current tillage erosion models are based on yield derived transport coefficients that are specific to the tillage tool being used. Other variables that can modify the transport coefficients include speed of operation and depth of tillage. The measurements of tillage coefficients in the field are time consuming and labor intensive. Because of these limitations determining variability of tillage coefficients has been difficult. A procedure that provides an internal estimate of variability that requires little extra labor or time is described. This method provides an indication of sampling error but does not address variability associated with operation of the tillage tool. In addition we present a modification of tillage tracer-soil separation that allows in-field processing. A method for estimating the movement of soil by tillage equipment was developed that utilized metallic washers and a magnetic sweep. The method allowed for a high retrieval rate of soil tracer (98%) for the plot treatments. The high retrieval rate and uncomplicated nature of the method allowed for the development of a regression equation describing the distance soil moves in the up and down direction through translocation by a chisel plow. The tillage translocation coefficient for the chisel plow was estimated to be 154 kg/m when operated at measured initial parameters. The initial parameter measurements included tillage speed, soil moisture content, soil bulk density, and soil texture. The derivation of the basic model for calculating tillage erosion rates and soil tillage translocation coefficient are also described.
EFFECT OF BOVINE VIRAL DIARRHEA VIRUS ON THE CHEMOKINE PRODUCTION OF MACROPHAGES

J. L. Gibbons, C. Chase and L. Braun
Department of Veterinary Science
South Dakota State University
Brookings, SD 57007

ABSTRACT

Bovine viral diarrhea virus is an important cause of respiratory and reproductive disease. BVDV is immunosuppressive and the mechanisms responsible have not been determined. In this project, four chemokines, important mediators of inflammation and innate immunity were measured using a BVDV in-vitro infection model. Bovine macrophages were isolated, cultured, and infected with three different strains of BVDV. The infected cells were harvested at 4 hrs and then total RNA was extracted. The RT-PCR analysis was used to determine the effect of BVDV infection of mRNA chemokine production of CCL-3 (MIP1-a), CCL4 (MIP-1b), CCL-5 (rantes) and CCL-11 (eotaxin) from macrophages. There were no measurable changes following BVDV infection. Chemokine production was not affected by BVDV infection in vitro.
RANGELAND DROUGHT MITIGATION BY BEAVER 
(*CASTOR CANADENSIS*) IMPOUNDED WATER

M. Striped Face-Collins and C.A. Johnston
Environmental Science
Sitting Bull College/United Tribes Technical College
Bismarck, ND
and
Department of Biology & Microbiology
South Dakota State University
Brookings, SD 57007

ABSTRACT

We investigated the role of beaver (*Castor canadensis*) as a keystone species in riparian zones of the Northern Plains. Beaver dams across Oak Creek, on the Standing Rock Reservation near Wakpala SD, retained water in the creek channel during July 2006 despite extreme drought; portions of the creek that were not impounded by beaver were dry. Water depth was measured at six cross-sections across a dammed portion of the creek, and volumetric calculations indicated that significant amounts of water were being stored in the beaver “pond.” Long-term discharge measurements at downstream U.S. Geological Survey gauging station 06354882 show that Oak Creek flow is highly variable, with minimum flows of 0 cfs and a maximum flow of 6,800 cfs that occurred on March 28, 1997. These findings substantiate anecdotal information gained from long-time area ranchers, which credited beaver-impounded water with saving their ranching operations from certain disaster during major drought conditions. By keeping the water in surface pools, beaver ponds mitigate a negative impact of climate change by making water available for use by livestock and other fauna that inhabit the riparian zone. Restoration of *Castor canadensis* to their rightful place in a balanced Northern Plains ecological system may contribute a significant portion of surface stored water, thereby locally mitigating the negative impact of current and future droughts and adverse climate changes on local ranching and wildlife management activities.
PREDICTING HABITAT SUITABILITY FOR LESSER SCAUP (AYTHYA AFFINIS) SPRING MIGRANTS IN EASTERN SOUTH DAKOTA

Sharon N. Kahara and Steven R. Chipps
USGS South Dakota Cooperative Fish & Wildlife Research Unit
Department of Wildlife and Fisheries Sciences
South Dakota State University
Brookings, SD 57007

ABSTRACT

Recent population declines of lesser scaup (Aythya affinis; hereafter scaup) have led scientists to hypothesize that habitat declines along spring migration routes may be contributing to arrival condition on breeding grounds. We used site-specific and landscape scale data collected from 2004 to 2006 to determine the variables that best described and predicted wetland use by scaup. Our results show that the availability and abundance of their preferred prey items, amphipods (Hyalella azteca and Gammarus lacustris) is more important than wetland size or depth, therefore scaup are more likely to select wetlands with high prey densities to optimize foraging efficiency. A predictive model developed using model averaged parameter estimates for submerged aquatic vegetation and amphipod abundance to predict scaup use performed well, explaining 64% of the variation in scaup use. Long-term amphipod data collection revealed that amphipod abundance in the spring was best explained by spring water depths ($F_{1.4} = 11.3$, $P = 0.02$). At the landscape scale, total wetland area and total shoreline length within 3000 m as well as high density development within 100 m of a site best described scaup use. By incorporating the effects of average monthly temperature and precipitation on site-specific and landscape scale habitat characteristics we were able to develop a temporally dynamic habitat suitability index model to determine the effects of climate change on availability of habitat for scaup spring migrants. Preliminary results indicate that changes in the local summer temperature and winter precipitation have led to declines in habitat suitability for amphipod which may in turn limit nutrient acquisition by scaup during the critical pre-nuptial migration period.
FLUCTUATION OF SOYBEAN FATTY ACIDS IN SOUTH DAKOTA ENVIRONMENTS

David Karki and Roy Scott
South Dakota State University
Plant Science, Brookings, SD 57007

ABSTRACT

Breeding to improve soybean oil quality has intensified over the past decade. Stability of performance across environments is essential to consistent production of modified fatty acid levels. This study was conducted in 2004 and 2005 growing seasons to determine we could maintain the modified fatty acid profiles in soybean across diverse South Dakota environments. Lines were evaluated for low linolenic acid. We studied the fatty acids most likely to fluctuate (oleic, linoleic, palmitic and stearic) with fluctuations in linolenic acid. Thirty-eight lines (maturity groups 0, I & II), derived from crosses of two high protein SD lines with two low linolenic IA lines were planted with three high yield checks, two low linolenic parents and two high protein parents. All lines were tested in seven eastern SD locations in two years. Location, maturity group, year and year x location effects were significant for all fatty acids except palmitic acid. None of the fatty acid showed significant entry x location effects. There were significant relationship between linolenic acid and palmitic acid, as well as oleic acid and linoleic acid. Regression coefficients (b) of 45 lines ranged from -0.5835 to 1.2412 and mean deviation (S’d) ranged from -0.518 to -0.056. These data indicated that soybeans with low linolenic acid content can be produced in northern environments without negative economic impacts.
DEVELOPMENT OF PORCINE INTESTINAL EPITHELIAL CELL CULTURE MODEL FOR ENCEPHALITOZOOON INTESTINALIS INFECTION

Gopakumar Moorkanat
Department of Biology & Microbiology
Center for Infectious Disease Research & Vaccinology

David Francis
Center for Infectious Disease Research & Vaccinology
Department of Veterinary Science

Michael Hildreth and Radhey S. Kaushik
Department of Biology & Microbiology
Center for Infectious Disease Research & Vaccinology
Department of Veterinary Science

South Dakota State University
Brookings, SD 57007

ABSTRACT

Microsporidiosis is an emerging and opportunistic infection especially in AIDS and cancer patients, organ transplant recipients and elderly people. Encephalitozoon intestinalis is an obligate intracellular parasite and the second most prevalent disseminating microsporidian infecting humans. E. intestinalis first infects enterocytes and further spread to various other organs including kidney, lungs and liver. No animal models closely related to humans and show clinical signs of this infection have been described so far. As a first step to develop a porcine infection model, we compared the infectivity of porcine and human intestinal epithelial cultures to E. intestinalis. Two porcine intestinal epithelial cell lines IPEC-J2 and IPEC-1 derived from jejunum and small intestine respectively, and a human colon cell line Caco-2 were grown in DMEM medium supplemented with fetal calf serum, insulin, transferrin, selenium, epidermal growth factor and antibiotics. A quantitative cell culture infectivity assay was used to compare the infectivity of different cell cultures each day up to 6 days post-infection. The method employed microscopical observation of intracellular spore masses after staining with optical brightener calcofluor white and a FITC-linked polyclonal E. intestinalis specific antibody. Both porcine and human cell lines showed significant infection with E. intestinalis by the second day. The infectivity and replication kinetics of E. intestinalis in both porcine and human cells were further compared and quantified using flow cytometry and real time PCR assays. The data support the hypothesis that E. intestinalis infects porcine intestinal epithelial cell cultures and pigs may prove as a good animal model for human E. intestinalis studies.
EXPRESSION OF TOLL-LIKE RECEPTORS IN THE PORCINE INTESTINAL EPITHELIAL CELL LINES

Matthew J. Anderson  
Department of Biology & Microbiology

Gopakumar Moorkanat  
Department of Biology & Microbiology  
Center for Infectious Disease Research & Vaccinology

Radhey S. Kaushik  
Department of Biology & Microbiology  
Center for Infectious Disease Research & Vaccinology  
Department of Veterinary Science

South Dakota State University  
Brookings, SD 57007

ABSTRACT

Intestinal epithelial cells play a significant role in mediating innate and adaptive immune responses in the gut. Intestinal innate mechanisms are recognized as central protective mechanisms against intestinal pathogens and include the barrier function of the epithelium, the presence of toll-like receptors (TLRs), and secretion of mucus, antimicrobial peptides and cytokines. TLRs are a type of microbial pattern-recognition receptors that recognize certain microbes and microbial components and induce signals to activate genes responsible for host defense. Bacterial peptidoglycan and lipoteichoic acid are recognized by TLR2; lipopolysaccharide by TLR4; flagellin by TLR5, and the CpG motif of bacterial DNA by TLR9. Intestinal epithelial cell lines which express various TLRs may represent the valuable and biologically relevant cell culture models for studying the TLR-mediated mechanisms. This study assessed the expression of TLR 2, -4, -5 and -9 in two intestinal porcine epithelial cell lines IPEC-J2 and IPEC-1 derived from jejunum and small intestine, respectively, from one day old pigs. The expression of TLR transcripts was quantified by real time reverse-transcriptase PCR. Both IPEC-J2 and IPEC-1 cells expressed comparable levels of TLR 2, -4, -5 and -9 transcripts. The presence of TLR proteins in both porcine cell lines was detected by immunohistochemistry using polyclonal antibodies against human TLRs. Both IPEC-J2 and IPEC-1 expressed TLR-2, -4 and -9 proteins but did not express TLR-5 protein. The findings of this study suggest that both IPEC-J2 and IPEC-1 cells may provide suitable porcine cell culture models for studying the mechanisms and modulation of TLR-mediated intestinal innate immune defenses.
HYPOGLYCEMIC EFFECTS OF MOMORDICA CHARANTIA IN DIABETIC ANIMAL MODELS

Kevin Ellis and Deig N Sandoval
Lakota Institute for Science and Technology
Natural Products Division
Oglala Lakota College
Kyle, SD 57752

ABSTRACT

Extracts from the tropical plant called Bitter Gourd Melon (Momordica charantia) have been used to determine the effectiveness on reducing sugar levels in the blood of diabetes induced laboratory animals. The animals were fed a high fat diet over a period of three months. Verification that the animals were diabetic was achieved by using measurements of the glucose and insulin levels in their blood. The information was analyzed using a mathematical model called Quicki. Even though, these studies were not statistically significant it has been determined that there is a glucose reduction in the animals’ blood. Over time we have seen that Momordica charantia extracts has actually lowered blood glucose values in the animal models. It has been shown that Momordica charantia does not promotes insulin resistance. The data obtained shows that there was about a 23% reduction in the glucose levels of the treated animals in about 70 minutes from the time of administering the extract to the time of measurement. Thus, the results showed that overtime the rats on high fat diet developed insulin resistance while the bitter gourd melon did not. Also, the insulin tolerance test suggests that bitter gourd melon had some effect in controlling large changes in the glucose levels in the blood of the treated animals.
DETECTING SHIFTS IN SOIL MICROBIAL COMMUNITY STRUCTURE AND FUNCTION POST LANDSPREAD OF MANURE OR BIOSOLIDS CONTAINING ANTIMICROBIAL CHEMICALS

Kelly Lehnert, Volker Brözel, Susan Gibson and Sharon Clay
Department of Microbiology
Department of Plant Science
South Dakota State University
Brookings, SD 57007

ABSTRACT

Soil microbial diversity and community interaction play an indispensable role in 2,4-dichlorophenoxyacetic acid (2,4-D) herbicide degradation. The addition of manure or municipal waste biosolids through landspreading may alter soil community structure and function if these materials contain antimicrobial chemicals like chlortetracycline (CTC), administered in livestock feed to promote animal growth and health or tetracycline (TET) utilized in human health. In this study, soil applied with manure collected from pigs fed standard CTC levels was compared to soil containing manure from control pigs fed no CTC, and a comparison of soil applied with biosolids containing TET or without TET to distinguish soil microbial shifts. Culturable aerobic heterotroph counts on R2A agar plates revealed unexpectedly high counts 7 days after treatment (DAT) of the CTC enriched applications. By 28 DAT, these counts were comparable to other treatments. It is unknown if the increase in culturable counts was due to native soil organisms or organisms present in manure. The density of 2,4-D degrading microorganisms using the Most Probable Number (MPN) method indicate increased growth of these degraders nearly 20 fold after adding 2,4-D to soil samples compared to MPN results of soil samples without the enhancement. Denaturing gradient gel electrophoresis (DGGE) analysis of polymerase chain reaction (PCR) -amplified 16S rDNA fragments from each soil sample allowed for representation of all microorganism present, culturable or not. Significant shifts in bacteria communities between the different manure treated soils are apparent. Future cloning and sequencing of specific DGGE bands will show the taxonomical diversity of the microbial community.
NEW VASCULAR PLANT RECORDS FOR NEBRASKA AND SOUTH DAKOTA

Grace M. Kostel, Ronald L. Hartman and B. Ernie Nelson
Rocky Mountain Herbarium
Department of Botany
University of Wyoming
Laramie, WY 82070

ABSTRACT

Additions to the vascular flora of Nebraska and South Dakota are reported herein. Past and recent inventories on the flora of the Oglala National Grassland (Nebraska) and the Buffalo Gap National Grassland (South Dakota) have resulted in 23 native or naturalized taxa new to these states or verification of reports. Much of this work is part of an ongoing inventory of the southern Rocky Mountains by the students and staff of the Rocky Mountain Herbarium.
CAPTOPRIL AND THE EFFECTS ON HIGH BLOOD PRESSURE

R.J. Hoff
Mount Marty College
Yankton, SD 57078

C. Kost and A. Stephenson
The University of South Dakota Medical School
Vermillion, SD 57069

ABSTRACT

High blood pressure (hypertension) results in cardiovascular disease, the leading cause of death in the U.S. Spontaneously hypertensive rats (SHR) are a genetic model of human hypertension. Our studies show that early treatment with a class of antihypertensive drugs known as ACE-Inhibitors prevents the development of hypertension in SHR and produces a long-term blood pressure (BP) reduction that persists after treatment is withdrawn. The mechanism for the persistent BP reduction is unknown, but may involve altered kidney function. This study was designed to evaluate the effect of ACE-Inhibitor treatment and its withdrawal on BP and kidney function in SHR.
GENES CONTRIBUTING TO THE INDUCED MULTICELLULARITY OF *BACILLUS CEREUS* GROWING IN SOIL

L. Weyrich, Y. Luo, J. Sutton, S. Vilain and V. Brözel
Department of Biology and Microbiology
South Dakota State University
Brookings, SD 57007

ABSTRACT

Bacillus, notably *B. cereus*, are readily isolated from a wild range of soils. When growing in soil or liquid extract of soil, *B. cereus* switches to a distinct multicellular phenotype. The ensuing bundles of chains are the basis for translocation of the species through soil by a process termed sliding. We have recently developed an *in terra* model system for studying the growth of bacteria in soil, using soil extractable soluble organic matter (SESOM). The aim of this research was to identify genes contributing to the soil-induced multicellularity of *Bacillus cereus* ATCC 14579. Random mutants were generated using LTV1 (Tn917) and screened for formation of rhizoidal colonies on SESOM agar. Mutated genes were identified by plasmid rescue. A number of mutants forming regular colonies or colonies spreading poorly were obtained, including a mutation in the purA gene, encoding adenylosuccinate synthetase. Interestingly some hyper-rhizoidal mutants were also found, including a mutant defective in the galE gene, encoding UDP-glucose 4-epimerase which transforms UDP-D-glucose to UDP-galactose, and a few other intriguing genes as well. This mutant yielded chains but no bundles or clumps, indicating a role in lateral adherence between cells.
MEASURING THE INFLAMMATORY CYTOKINE EFFECT OF BOVINE VIRAL DIARRHEA VIRUS ON PERSISTENTLY INFECTED CATTLE

L. E. Kattelmann, L. J. Braun and C. C. L. Chase  
Department of Veterinary Science  
South Dakota State University  
Brookings, SD 57007

ABSTRACT

The purpose of this project was to observe inflammatory cytokine effects in cattle persistently infected with Bovine Viral Diarrhea Virus (BVDV). The working hypothesis was that persistently infected animals produce higher levels of immunosuppressive cytokines. The cytokines tested include interleukin-10 (IL-10), tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-α), and interleukin four (IL-4). Cytokine levels in persistently infected (PI) animals were compared to age-matched healthy animals in vivo and in vitro. MDM and Sera were collected monthly for a three-month period, and cytokine levels were determined using Enzyme-Linked immunosorbent Assays (ELISA). The IL-4 levels in the sera of the PI herd were higher than the healthy animals throughout three-month period. The TNF-α levels decreased as the disease progressed in the animals. The IL-10 stayed high through the three months compared to the healthy animals. This indicates that these animals are under the influence of anti-inflammatory cytokines.
HABITAT-BASED DISTRIBUTION OF BREEDING LANDBIRDS AT CUSTER STATE PARK IN THE BLACK HILLS OF SOUTH DAKOTA

M.R. Schickel and C. Dieter
Department of Biology/Microbiology
South Dakota State University
Brookings, SD 57007

ABSTRACT

A significant nationwide decline in the abundance of many migrant bird species has been correlated to the decline of existing habitat or quality of habitat needed throughout the year. The objectives of this study were to construct a complete list of landbird species breeding within Custer State Park (CSP) along with their abundance and habitat-use relationships during the breeding season. We surveyed nocturnal breeders by vehicular point count during 2005-2006 winters, and surveyed songbirds by point transect from 2004-2006, pre-stratified by four major habitat types (mixed-grass prairie, coniferous forest, post-fire burn, and deciduous corriors). Species density estimates were calculated using Program Distance, habitat measurements were analyzed using canonical correlations analysis (CA), and pooled transect data was compared to plot-wise data using Kruskal-Wallis to analyze edge effect on species richness. Confirmed breeding by 123 species of birds was documented in CSP during the 2004-6 survey seasons. Twenty-two species were observed using all major habitat types in the park. Plot-wise species richness was significantly higher than pooled data in highly fragmented areas such as post-fire burn (p=0.018). Riverine, marsh, aspen/birch woodlands and shrublands were found to increase species richness in the park despite covering a small percentage of total land area. Thirty species of management concern in the Black Hills including red-headed woodpecker and McGillavry’s warbler used these resources in CSP during 2004-2006, providing baseline information to CSP biologists for future management decisions.
ECOLOGY OF NORTHERN FLYING SQUIRRELS (*GLAUCOMYS SABRINUS*) IN THE BLACK HILLS, SOUTH DAKOTA

Melissa J. Hough and Charles D. Dieter
Department of Biology/Microbiology
South Dakota State University
Brookings, SD 57007

ABSTRACT

There is limited information and research on the ecology of the isolated population of northern flying squirrels, *Glaucomys sabrinus bangsi* (Rhoads) in the Black Hills of South Dakota. From May through August 2005 and 2006 we captured flying squirrels with Tomahawk and Havahart live traps throughout the Black Hills. Between the two years, we anesthetized 20 female and 25 male flying squirrels with Halothane and then fitted them with radio collars. We examined home range, habitat use, and denning behavior of the collared squirrels. Males averaged larger home ranges than females [minimum convex polygon (MCP)]; 13.6 hectares (range=3.9-37.4 hectares) and 7.5 hectares (range=2.5-20.0 hectares), respectively. Flying squirrels in the 2006 study area averaged larger home ranges than the flying squirrels in the 2005 study area, 15.3 hectares (range=2.5-14.8 hectares) in 2006 and 6.7 hectares (range=4.5-37.4 hectares) in 2005. Using radio tracking information, we created GIS habitat models for flying squirrels in the Black Hills to determine differences in habitat use between the two study areas. The habitat characteristics associated with 107 dens used by flying squirrels was compared to available tree within the flying squirrels’ home ranges. Snags were selected more than expected based on availability (p<.001). Flying squirrels select cavities in snags, as well as live aspen (*Populus tremuloides*) and birch (*Betula papyrifera*), more than dray (external) nests. These results on home range, habitat use and denning behavior will assist wildlife professionals in the management of flying squirrels in the Black Hills.
GENETIC POPULATION STRUCTURE OF THE FINESCALE DACE, *PHOXINUS NEOGAEUS* AND THEIR HYBRIDS

Jake Miller, Cynthia Anderson, Shane Sarver
Black Hills State University
Spearfish, SD 57799

ABSTRACT

The finescale dace, *Phoxinus neogaeus* is a small minnow (Family Cyprinidae) that occurs from northwestern Canada to New England, south into northern Minnesota, Wisconsin, Michigan and New York. Isolated populations have been reported in North Dakota, South Dakota, Nebraska and Wyoming. This species is listed as state endangered in South Dakota, as state threatened Wyoming and Nebraska, and as a sensitive species in US Forest Service Region 2. Little is known about the life history of the species except that it lives in small, weedy streams or ponds and is often found with the northern red-belly dace, *Phoxinus eos* with which it will hybridize. Hybrids are capable of asexual reproduction by gynogenesis, where diploid clonal females produce unreduced ova containing an exact copy of the maternal *eos/neogaeus* genome. Such hybridization is a contributing factor to the demise of *P. neogaeus* populations. Additionally, poor records concerning the historic distribution of finescale dace in the Great Plains region makes it difficult to ascertain the extent of population decline in this region. Here we present the development of nuclear microsatellite markers by creating a microsatellite enriched genomic library for *P. neogaeus*. These markers will be used to assess the current population structure and the extent of hybridization in the Great Plains portion of the species range. Information from this research can be used to elucidate some historical trends leading to a greater understanding of the biogeography of this species in the Great Plains and will provide information regarding the genetic status of this locally rare species to fishery managers in order to devise effective management strategies.
INFLUENCE OF PARTIAL TANK COVERS
ON THE AGGREGATION BEHAVIOR
OF JUVENILE LARGEMOUTH BASS
DURING HATCHERY REARING

Nathan S. Richards
South Dakota Department of Game, Fish, and Parks
Blue Dog State Fish Hatchery
Waubay, SD 57273

Michael L. Brown
Northern Plains Biostress Laboratory
Department of Wildlife and Fisheries Sciences
South Dakota State University
Brookings, SD 57007

Michael E. Barnes
South Dakota Department of Game, Fish and Parks
McNenny State Fish Hatchery
Spearfish, SD 57783

ABSTRACT

Partial tank covers were evaluated for their influence on the behavior of juvenile largemouth bass *Micropterus salmoides* during intensive hatchery rearing. Partial covers utilized during hatchery rearing have been shown to increase the growth and performance of several salmonid species; thus, improving their post-stocking success. Similarly, if largemouth bass utilize partial covers there is potential for increased growth and performance, which increases their chances of post-stocking survival. Largemouth bass were reared in rectangular concrete raceway tanks (1.72 m$^3$) that were either completely open (controls) on top or partially (31%) covered. Largemouth bass reared in partially covered tanks utilized the additional cover. There was a significantly ($t=13.66$, $P=0.004$) higher density of bass located underneath the additional cover (582/m$^3$, ±13.5, n=2) when compared to controls (353/m$^3$, ±9.99, n=2). Because our largemouth bass were reared on 10.5°C well water we were unable to document the influence of partial tank covers on the growth and performance of largemouth bass. However, we plan to look at these effects during the summer of 2007.
BIDIRECTIONAL ARTIFICIAL SELECTION FOR AEROBIC CAPACITY IN RESULTS IN A CORRELATED RESPONSE IN WHEEL-RUNNING ACTIVITY

University of South Dakota
Vermillion, SD 57069

ABSTRACT

A positive genetic relationship between aerobic capacity and voluntary exercise activity levels has been suggested from earlier studies of mice selected for increased wheel-running activity. To further investigate the proposed relationship between aerobic capacity and exercise behavior in another animal model, wheel running activity was studied in rats bidirectionally selected for differences in intrinsic aerobic capacity (high capacity runners – HCR; low capacity runners – LCR). Each rat was housed individually with access to a running wheel and wheel-running activity was recorded for 6 weeks to determine differences in voluntary activity levels. HCR animals exhibited 37% greater total wheel-running distance per day than the LCR (LCR- 11.7km/day vs. HCR- 15.7km/day) due to a 15% increase in wheel-running speed (36.5m/min versus 41.0m/min) combined with a 22% increase in running duration (1968.6min/day versus 2386.1min/day). Total wheel running was significantly different between LCR and HCR (t_{55} = -11.09; P<0.001). Differences in the intermittency of wheel-running were also observed. HCR animals engaged in 10% more bouts of running per day (31.1 vs 34.2 meters), ran 19% faster during episodes of running (36.0 vs. 41.3 meters/minute), and 21% longer distances than LCR animals (342.8 vs 421.5 meters/bout). These results are consistent with earlier work that suggests a phylogenetically conserved relationship between physiological capacity and behavioral activity for aerobic performance.
SOCIALLY MEDIATED FEAR LEARNING IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

Russ E. Carpenter  
Department of Biology  
Cliff H. Summers  
Department of Biology  
Neuroscience Group, Basic Biomedical Sciences  
University of South Dakota  
Vermillion, SD 57069

ABSTRACT

Rainbow trout in captivity interact aggressively, and form distinct social hierarchies. The most aggressive fish, as measured by decreased latency to attack, become dominant. The increase in aggression from dominant individuals towards subordinate fish leads to a number of deleterious behaviors such as decreased food intake, less favorable water column position, decreased metabolic rate and an incentive to escape. Increased anxiety induced by the neuropeptide corticotrophin releasing factor (CRF) stimulates an increase in attacks, but concurrently stimulates increased retreat and escape behavior. Mammals in similar anxiety producing trials have the capacity to learn specific spatial tasks such as those associated with the Morris water and radial arm mazes. We hypothesized that fish have a similar capacity to learn spatial tasks under anxiogenic conditions. We further hypothesized that dyadic social interaction against a substantially larger opponent provides the impetus as well as the necessary information to stimulate goal-oriented learning in the subordinate individual. That is to say we believe that social stress and aggression stimulate learning. In a flow through fish tank of 70 gallons, 4 equally sized compartments were created using opaque Plexiglas. A small (~100 g) juvenile rainbow trout was placed in one of the center compartments, and a large (~300 g) fish was placed in the adjacent center compartment. Behind the outer wall of the small fish’s tank, a second piece of Plexiglas was in place that contained a small (2 inch diameter) hole leading to an empty compartment. This escape hole was large enough for the smaller, but not the larger fish to pass through, and was only available when the larger fish was present. Similar to the Morris water maze, the smaller fish had to find this hole in order to obtain relief. However, in this case the task was three-dimensional and the stressor is social rather than physical. Once a day the water inflow to the tank was turned off; 15 seconds later the center divider was removed and the fish were allowed to interact for 15 minutes, or until the small fish escaped. The larger fish were very aggressive, and the learning curve for subordinate fish was dramatic, with an approximately 600% improvement in escape time over seven days. The fish learn very rapidly, improving 400% in the first two days. Escape time im-
proved daily until fish escaped in just less than two minutes for the final three trials. Plasma samples for cortisol measurement were taken 3 days before and 1 day after social interaction and learning trials. For the samples taken after the trials, fish were presented with the inflow water off but no large fish challenge. Fish learning to escape using the opening as a passageway showed no significant elevation in plasma cortisol. However, fish that did not learn to escape exhibited a four-fold increase in plasma cortisol, even though no large fish was presented as a social challenge. Elevated cortisol levels may represent a slow return to baseline after repeated social defeat. However, inflow water off has been demonstrated to be a sufficient conditioned stimulus to provoke increased plasma cortisol concentrations. Elevated plasma cortisol levels may therefore represent fear conditioning in non-escaping fish, demonstrating learning in these fish as well.
SYNTHESIS OF 3-METHYL-4-(1-METHYLETHENYL) PYRROLIDINE ON A SOLID SUPPORT

Ryan Hajek, Ryan Esser and Nandeo Choony
Chemistry Department
Mount Marty College
Yankton, SD 57078

ABSTRACT

An attempt was made to synthesize the cycloadduct 3-methyl-4-(1-methyl ethenyl)pyrrolidine involving an intramolecular ENE cycloaddition reaction on a solid support polymer bound triphenylchloromethane resin which acted as a steric buttress. After it was made on the resin, it was isolated from it by deprotection with a dilute acid. This cycloadduct is a constituent of many neuroactive compounds and hence can be used as a template in the pharmaceutical industry. After characterization of the end-product, it was reasonable to conclude that the desired product was formed on the solid phase.
DESIGNING A BETTER SOYBEAN MEAL FOR NON-RUMINANT LIVESTOCK

R. Scott, B. Klein, and D. Karki
Plant Science Department
South Dakota State University
Brookings SD 57007

ABSTRACT

Soybean [Glycine max (L.) Merr.] seeds store phosphorous mostly as phytic acid, which is not nutritionally available to non-ruminant livestock. Excess un-utilized P in manure is a pollutant. The objective of this study was to develop soybean with reduced phytic acid, and examine the effects of the low phytate genes on agronomic and quality traits in near isogenic populations with and without the Monsanto Roundup Ready® genes which confers glyphosate herbicide tolerance. Soybean genotypes that were different only in their ability to tolerate glyphosate herbicide were crossed to the same low phytate donor parent to develop two sets of genotypes. After confirming the presence of the low phytate genes in the resulting genotypes, protein, oil, yield, and other agronomic and quality traits were compared within and among the two populations. Protein concentrations ranged from 350-430 and oil 140-180 grams per kilogram among 36 lines without glyphosate tolerance. Similar ranges of protein (330-470) and oil (130-180) were found among 143 glyphosate-tolerant genotypes. Stronger negative relationships were found between yield and protein among glyphosate-tolerant (r= -0.47, p<0.05) than glyphosate-susceptible (r= -0.32, p<0.05) lines. This was also true of the relationships between protein and oil (r= -0.76, p<0.01 and r= -0.55, p<0.05, respectively) for glyphosate-tolerant and susceptible populations. There were 11% of the glyphosate-tolerant lines with yields at least 90% of the mean of three high yield checks and protein at least 400 grams per kilogram, compared to 5% for glyphosate- susceptible lines. Presence of the low phytate genes did not hinder yield and protein improvement, but restricted the ability to maintain desirable oil concentrations.
COMPARATIVE ECOLOGY OF NATIVE AND NON-NATIVE *PHRAGMITES AURALIS* (COMMON REED) GENOTYPES

M. G. Tulbure, D. M. Ghioca, D. F. Whigham, and C. A. Johnston  
Department of Biology and Microbiology  
South Dakota State University  
Brookings, SD 57007

ABSTRACT

*Phragmites australis* is one of the most invasive species in wetlands along the Atlantic coast. The non-native M genotype is responsible for the spread of the species. We conducted a survey of *Phragmites* stands in the Chesapeake Bay watershed (MD, USA) in both freshwater and brackish wetlands. We did not find any native stands in brackish wetlands. We compared biomass, stem density, nutrient (nitrogen and phosphorus) resorption proficiency and radial oxygen loss in three pairs of native and non-native *Phragmites* stands during the summer of 2006. Native and non-native stands in each pair were located adjacent to each other along King’s Creek near Easton, Maryland. Biomass, green, old and total stem densities were significantly higher in non-native than native stands. Nitrogen concentration in senesced leaves was significantly higher for the non-native genotype, while no significant differences were noted in phosphorus concentrations. This suggests that the native genotype has a higher nitrogen deficit than the non-native genotype. Ventilation efficiency parameters such as gas flow rates and gas flow rates per generated pressure differentials were not significantly different between the two genotypes. However, when the flow rates were related to the corresponding shoot densities, the non-native stands with higher densities achieved higher ventilation efficiency (significant at p<0.1) per 25cm$^2$ of reed. The higher oxygen loss of the non-native stands could benefit the plant by increasing the nitrification of ammonium, oxidizing potentially toxic compounds in the rhizosphere and oxidizing sulfides, which may favor plant growth in brackish wetlands with higher sulfide concentrations.
MUC13 EXPRESSION AND ITS IN VITRO FUNCTIONS IN OVARIAN CANCER

Kelley Vannatta, Namita Vinayek, Akira Watanabe, Katrina Dunham, Maria C. Bell, Michael D. Koch, Hiroyuki Aburatani, Meena Jaggi and Subhash C. Chauhan
Cancer Biology Research Institute
Sanford Research/USD
Sioux Falls, SD 57105

ABSTRACT

Mucins are attractive targets for cancer diagnosis. MUC13 is a recently identified mucin and is suggested to be over-expressed in ovarian cancer. We analyzed the expression profile and function of MUC13 to determine its role in ovarian cancer. A monoclonal antibody (clone PPZ0020) was used to determine the expression profile of MUC13 by immunohistochemistry, using an ovarian tissue microarray slide containing normal and cancerous samples. Additionally, MUC13 expression was also analyzed in 55 clinically proven ovarian tissue cancer samples. For functional analysis, a full-length MUC13 was exogenously expressed in a MUC13 null ovarian cancer cell line SKOV-3 and evaluated by means of cell proliferation and migration assays. Paired student t-tests were performed for statistical analysis. MUC13 expression was undetectable in normal and benign ovarian samples and was significantly (p<0.005) higher in cancer samples. MUC13 expression was most frequent in mucinous types of ovarian cancer samples. Additionally, in functional studies the exogenous expression of MUC13 in SKOV-3 ovarian cancer cells increased cellular migration, cell proliferation, and the number of cells in S-phase of cell cycle. Furthermore, MUC13 expression reduced cell to cell aggregation. These results indicate that MUC13 is aberrantly expressed in ovarian cancer and may have a role in ovarian cancer pathogenesis.
NANOSCALE ZIRCONIUM TUNGSTATE SYNTHESIS AND USE AS A FILLER FOR DIMENSIONAL STABILITY

Steven Schnabel, Lidvin Kjerengtroen, William Cross, Jon Kellar and Wayne Weyer
South Dakota School of Mines and Technology
Rapid City, SD 57701

ABSTRACT

Previous research by our group has shown that addition of an isotropic negatively thermal expanding material to a polymer results in a matrix with near dimensionally stable properties. Eshelby models have shown that the morphology of the filler material can affect the overall composite coefficient of thermal expansion. Toward this end, particle shape control of zirconium tungstate (ZrW₂O₈) has been examined by an inverse micelle synthesis. Diffraction patterns clearly indicate formation of 65-100% crystalline zirconium tungstate with remaining traces of tungsten oxide (WO₃). Rod shaped particles having diameters of ~100-200 nanometers and lengths of one to three microns were found. The size of the resulting particles is smaller than compared to those of sol-gel processing techniques, which have also been evaluated. The details associated with the inverse micelle preparation of this filler will be reported as well as the affects of different surfactants and temperature on the particle morphology and size. Finally, the nanoscale zirconium tungstate filler was incorporated into polymer matrix and the composite coefficient of thermal expansion determined as a function of temperature.
STABILITY OF PASSIVATED ACID ROCK AFTER INTENSIVE ROOT SYSTEM EXPOSURE

C.E. Werkmeister, D.D. Malo, T.E. Schumacher and J.J. Doolittle
Plant Science Department
South Dakota State University
Brookings, SD 57007

G.C. Miller
Natural Resources and Environmental Sciences
University of Nevada
Reno, NV 89557

ABSTRACT

The durability of a potassium permanganate protective coating (passivation) on potentially acidic waste rock was examined for preventing acid generation. There is limited biological and physical data on the environmental impact and durability of passivation technology. The objective was to determine if exposure of passivated acid waste rock to repeated cycles of intensive root growth would affect coating stability. Passivated treatments were compared to limed waste rock in columns with and without plants. Passivation stability was determined by measuring the pH, EC, iron, and sulfate of drainage leachate and saturated paste extracts, and by the use of a hydrogen peroxide stability test. The treatments were kept at field capacity and were leached once a month with RO water. The stability study showed 1) no root system effects on passivation stability; and 2) no difference in expression of potential acidity of waste rock between passivation and liming of 15%, by weight of waste rock.
ABSTRACT

The ability of the fumaric acid-producing fungal strains *Rhizopus oryzae* ATCC 10260 and ATCC 20344 to utilize corn distillers grains with solubles as a substrate for fumaric acid production were compared. The commercial applications for fumaric acid include its use in foods, beverages, paper sizing and printing inks. After inoculating untreated corn distillers grains with solubles, corn distillers grains with solubles treated by sterilization or corn distillers grains with solubles treated by acid hydrolysis and sterilization with the fungal strains, the solid-state fermentation of the grains occurred for 240 hours at 25°C. Subsequently, the grains were processed and the resultant supernatants were assayed for their fumaric acid contents. It was observed that *R. oryzae* ATCC 10260 and ATCC 20344 produced fumaric acid on the untreated and treated distillers grains. At least a 1.4-fold higher concentration of fumaric acid was produced by ATCC 20344 on the untreated and treated distillers grains than was produced by ATCC 10260. Overall, *R. oryzae* ATCC 20344 was able to produce fumaric acid more effectively than ATCC 10260 on corn distillers grains with solubles as a substrate using solid-state fermentation.
DISTRIBUTION, DENSITY, AND VIABILITY OF THE SOIL SEEDBANK OF A FERAL POPULATION OF ALFALFA (MEDICAGO SATIVA SUBSP. FALCATA) IN MIXED-GRASS PRAIRIE

Lan Xu
Department of Biology and Microbiology

Arvid Boe
Department of Plant Science

Patricia S. Johnson and Roger N. Gates
Department of Animal and Range Sciences

South Dakota State University
Brookings, SD 57007

ABSTRACT

Distribution, density, and viability of a soil seed bank can provide insight into various aspects of ecology and population dynamics, in particular, the potential for a species to disperse, establish, and persist in a plant community. Our objectives were to determine spatial distribution pattern and viability of the seed bank of a feral alfalfa population, and to examine the relationship between the density of the alfalfa seed bank and plant communities in the native mixed-grass prairie. The study was conducted on the Grand River National Grassland in SD during 2004 through 2006. Two sites where alfalfa distribution has been concentrated were sampled. Two permanent transects were established on each site along environmental gradients. Distinctive plant communities were identified along each transect and between two transects areas along the environmental gradients in each site. In each community, ten 0.25 m² quadrats were randomly located and cover by species was recorded. Within each quadrat, three soil seed bank samples were extracted using a bulb planter (5 cm dia. X 7.5 cm depth). Alfalfa seeds were removed from soil and counted. Germination and viability were determined by AOSA testing procedures. Spatial distribution of alfalfa seed bank was associated with plant communities, which varied with topographic position, soil texture, and soil moisture gradients. Alfalfa seed density was strongly correlated with alfalfa cover. The highest alfalfa seed density was more than 39,000 seeds m⁻², which is about 790 kg ha⁻¹. Greater than 99% of alfalfa seeds collected from the soil seed bank were viable.
MACROINVERTEBRATES IN THE LITTORAL OF A PRAIRIE POTHOLE, OAK LAKE, BROOKINGS COUNTY

Nels H. Troelstrup, Jr. and Kristopher Dozark
Department of Biology & Microbiology
South Dakota State University
Brookings, SD 57007

ABSTRACT

Littoral zones are near-shore areas along the perimeter of a lake basin capable of supporting rooted macrophytic vegetation. These are some of the most productive habitats on Earth and focal points of high biodiversity. The objective of this effort was to characterize macroinvertebrate communities within the littoral of an eastern South Dakota prairie pothole. Oak Lake is an intermittently exposed prairie pothole (163 ha) located in northeastern Brookings County, South Dakota. Macroinvertebrate samples were collected from sites around the basin perimeter during four separate projects extending from 1994 to 2006. Samples were collected with a tube sampler in 1994, a standard D-frame net from 1997 to 2000 and a petite net in 2005 and 2006. A total of 212 unique invertebrate taxa were collected, representing 5 phyla, 11 classes, 31 orders, 87 families, and 192 genera. Diptera (Chironomidae: Insecta) (44) contributed the greatest number of genera followed by Trichoptera (20), Coleoptera (16), Hemiptera (16) and Haplotaxida (15). Slightly more than one-quarter of unique taxa were burrowers. However, climbers (15%), clingers (13%), sprawlers (17%) and swimmers (15%) were evenly represented. Most invertebrate genera were gathering-collectors (33%) or predators (37%). Tolerance values to organic pollution ranged from 1-10 and averaged 6.59 among all unique taxa. Results of this effort demonstrate the high taxonomic and functional diversity of a relatively undisturbed prairie pothole littoral invertebrate community. Additional studies are needed to inventory and describe the regional biodiversity within these systems in support of long-term monitoring and management efforts.
NON-RADIOACTIVE THIAMINASE ASSAY

Jessica Partridge and Micheal Zehfus
College of Arts and Sciences
Black Hills State University
Spearfish, SD 57799

Mike Barnes
South Dakota Department of Game, Fish and Parks
McNenny State Fish Hatchery
Spearfish, SD 57783

ABSTRACT

Recent studies in North American salmonids (lake trout), have suggested that thiamine (vitamin B₁) deficiency may be a cause of early mortality syndrome (EMS). This deficiency is thought to be based on the consumption of smaller forage species containing thiaminase, an enzyme that destroys thiamine.¹ The destructive effects of thiaminase can be seen in various fish populations of the Great Lakes, as well as the Oahe Reservoir in South Dakota. This study reports on the development of a non-radioactive assay of thiaminase activity. It is currently possible to analyze thiaminase using a radioactive assay method, however, this method requires radioactive materials, and can only be performed in labs with a radioactivity license. The non-radioactive assay we are developing eliminates this requirement and allows greater access towards identifying the factors contributing to EMS in our lakes.

The new assay is based on the radioactive procedure, as provided by USGS Biochemist Jim Zajicek². In this method excess nicotinamide and radioactive thiamine are added to whole fish extract. If thiaminase is present, the thiamine in the tube is degraded to radioactive 4-methyl-5-thiazolylethylacetate (TE). The radioactive TE is then extracted into ethyl acetate and the radioactivity of the TE extract is measured with a scintillation counter. The amount of radiation in the extract is a direct measure of how much TE was released by the fish extract.²

In our non-radioactive assay we follow the same general procedure, but extract the TE out of the assay tube by filtering it through a carbograph solid phase extraction (SPE) column. The TE is released from the column by washing it with an organic solvent. After concentrating the organic solvent by evaporation, the concentrate of TE in the sample is high enough that it may be measured directly using a flame ionization detector (FID) on a gas chromatograph. This research will greatly contribute to understanding the causes of EMS in lake trout and walleye and allow larger studies of thiaminase activity to be carried out.

¹ D. Honeyfield; J. Hinterkopf Isolation of Thiaminase-Positive Bacteria. 2002
² J. Zajicek Thiaminase I Assay. 2002
ABSTRACT

The Cyprinidae are a group of fishes with highly developed gustatory systems. Most notable among these are the goldfish and carps, which possess elaborate neural structures in the hindbrain that receive gustatory input from numerous taste buds in the oropharyngeal cavity. This sensory input travels through the facial nerve from the rostral oral cavity and through the glossopharyngeal and vagal nerves from the pharynx. The gustatory afferents terminate in highly organized enlargements of the dorsal medulla called the facial, glossopharyngeal, and vagal lobes, respectively.

We start by studying selected branches of the glossopharyngeal (IX) and vagal (X) nerves of Zebrafish. These nerves run from the oropharyngeal cavity to the vagal lobe of the brain. Zebrafish are minnows (cyprinids) and their vagal lobe is not as highly developed as the vagal lobe found in suckers (catostomids). Cyprinids are found in tropical bodies of water and catostomids are found in waters ranging from large lakes and rivers to small, mountainous headwaters. This brings about questions as to the correlation between the evolutionary characteristics of the vagal lobe and the ecological niches of the different fish.

We begin by using a post-mortem neuronal label, DiI, to trace the pharyngeal afferent neurons in Zebrafish. Once the nerves have been labeled, we will wait 7-14 days for intracellular diffusion of the label. The brains are then removed and sectioned at 50 μm to analyze using fluorescent microscopy. By tracing the DiI we can identify the sites of termination of the afferents as well as the organization of efferents and other projection neurons in the vagal lobe. This data will contribute to a larger study of evolution and the function of the vagal lobe.
EXPLORATION OF A GREEN SUPPORT FOR NITROALDOL CONDENSATION

Anders J. Davidson, Alex C. Johnson,
Katie L. Severson and Jetty Duffy-Matzner
Augustana College
Sioux Falls, SD

ABSTRACT

The classic Henry Reaction, a nitroaldol condensation, is notoriously problematic. It is driven by a strong base, whose presence initiates unwanted side reactions and results in poor yields. Additionally this reaction requires long reaction times, solvent use, and difficult extractions. Thus an alternative method would be desirable in the synthesis of nitroalcohols. Recent literature\textsuperscript{1-4} states that excellent yields are achieved when nitroaldol condensations occur in the presence of an aminosilane-treated solid support. This process reduces many complications of traditional Henry reactions by minimizing side reactions, shortening reaction times, eliminating solvent use, and simplifying product isolation. Thus, the solid support provides a simpler and greener synthesis of nitroalcohols. The goal of this work is to synthesize and explore an aminosilane that, when attached to commercially available silica gel, promotes the nitroaldol condensation for both $\alpha$-branched and linear aldehydes.

\textsuperscript{3} G. Demicheli; et al. Tet. Let. 2001, 42, 2401-2403.
LOCATION AND CHARACTERISTICS OF SPRINGS CONTRIBUTING WATER TO THE OAK LAKE BASIN, BROOKINGS COUNTY, SD

Eric Dominiack and Nels H. Troelstrup, Jr.
Department of Biology & Microbiology
South Dakota State University
Brookings, SD 57007

ABSTRACT

Prairie pothole basins receive water directly from surface and groundwater sources. Spring sources to some of these basins may be significant and present unique habitats. The objectives of this study were to (1) locate surface spring drainages into the Oak Lake basin and (2) characterize physical, chemical and biological characteristics of these spring habitats. Ten spring sources were located using a GIS and sampled weekly from June 7 to August 21, 2006. Discharge was estimated from measurements of channel width, depth and velocity. Water temperature, conductance, pH and dissolved oxygen were measured from mid-channel using a multi-parameter probe. Macroinvertebrates were collected from each spring with a petite net (500 um mesh) on June 7. Cumulative discharge from the ten springs ranged from 3045 cm$^3$/sec to 5604 cm$^3$/sec. Nine of the ten springs were flowing at the beginning of the sampling period but only four were still flowing by August 21. Discharge from flowing springs contributed more water to the basin than was discharged from the lake outlet. Discharge, water temperature and chemistries varied significantly among the ten spring sites. Invertebrate collections were comprised of Coleoptera, Diptera, Trichoptera, Crustacea, Mollusca and Oligochaeta. Total invertebrate abundance ranged from 114 to 308 individuals per sample. Diptera were numerically most abundant from all spring sites followed by Oligochaeta. Gathering-collectors were the most abundant feeding guild. Data from this effort demonstrate significant surface water contributions and unique biological communities within these poorly studied habitats.
DEVELOPMENT OF AN OPTIMAL MACROINVERTEBRATE BIOASSESSMENT INDEX FOR PRAIRIE LAKES IN NORTHEASTERN SOUTH DAKOTA

R.W. Vander Vorste and David German
South Dakota State University
Water Resources Institute
Brookings, SD 57007

ABSTRACT

Biological monitoring is a necessary tool used for assessing the quality of fresh water ecosystems. Accurate biological assessments allow for determining the exposure of aquatic communities to different classes of stressors (e.g., domestic waste, agricultural runoff and sedimentation, pesticides, habitat alteration). Biomonitoring can guide managers toward the proper preventative or restorative actions for impaired ecosystems. Despite the widespread use of multimetrics for bioassessment in streams, little work has been published on multimetric indices for lakes.

This study examined the macroinvertebrate communities and water quality of Enemy Swim Lake, Clear Lake and Lake Minnewasta in northeastern South Dakota during May-September 2006. The macroinvertebrate communities were collected using sweep-nets, Hester-Dendy subsamplers, and Eckman dredges and individuals were identified to genus. Taxa richness and composition, feeding habits and habitat use were used to develop metrics. Metric scores were compared to the water quality status of the lake. Trophic status of the lakes correlated with metric scores. These lakes ranged in trophic status (TSI) from mesotrophic (moderate levels of nutrients) to hypereutrophic (high levels of nutrients) during the sampling period. Enemy Swim was found to have the highest water quality based on TSI and received high scores in metrics related to taxa diversity and pollution intolerance. Clear Lake had intermediate water quality and metric scores. Lake Minnewasta had the lowest water quality based on TSI scores and also the lowest metric scores out of the three lakes in the study.
GENE EXPRESSION IN ECOLOGICALLY MEANINGFUL CONTEXTS: EVOLUTION OF PLANT DEFENSE IN COMPETITIVE ENVIRONMENTS

Riston Haugen and David H. Siemens
Biology
Richard Gayle
Mathematics
Black Hills State University
Spearfish, SD 57799

ABSTRACT

Plants in the wild are attacked by herbivores and pathogens and often grow next to other plants that represent potential competitors. Therefore, in some cases one would expect the simultaneous evolution of defense and competitiveness in plants. However, the optimal defense hypothesis predicts a tradeoff between these factors. Plants that compete effectively against neighbors are expected to have lower defense levels because growth diverts limited resources away from production of defensive compounds. In contrast to these predictions, recent studies have found evidence that some plant species may be able to simultaneously compete and defend effectively. One hypothesis for this result is that some defensive traits, such as toxin concentration, have dual functions in defense and competition.

In two growth room experiments in which we examined glucosinolate (plant toxin) concentrations and transcript profiles of Boechera stricta, a close wild relative of Arabidopsis thaliana, we tested (1) whether neighboring plants elicit defense responses, and (2) whether there was overlap in gene expression patterns between herbivory and competition treatments. In one experiment involving three treatments (herbivory, competition, control) we observed over 500 significantly differentially expressed genes and evidence that competition elicited genes with known function in defensive pathways.

Key Words

Fenomics, microarray, Optimal Defense Hypothesis
BLOCK PREDATOR MANAGEMENT IN NORTHEASTERN SOUTH DAKOTA

N. R. Docken and C. D. Dieter
Department of Biology and Microbiology
South Dakota State University
Brookings, SD, 57007

ABSTRACT

The waterfowl rich and productive Prairie Pothole Region (PPR) of North America has seen decades of decline in duck nesting success, dropping to a 10% average in the 1990’s (Chodachek 2003). These losses can be attributed to loss of habitat, increased agriculture, land fragmentation, and increased predation on nesting waterfowl (Garrettson and Rohwer, 2001). While conservation efforts such as the Conservation Reserve Program (CRP) have slowed losses of nesting habitat, waterfowl managers have been concerned about nest predators. Predation is a main source of waterfowl mortality during spring with more than 70% of nest losses attributed to predation (Sargeant and Raveling 1992). The Delta Waterfowl Association has been studying predator removal through trapping on township size tracts of land since the early 1990’s and have found that nest success can be significantly increased (Garrettson and Rohwer, 2001). Predator removal may also enhance ring-necked pheasant (Phasianus colchicus) nesting success, but there has been no research on this subject.

In this two-year study, we will be searching for and monitoring duck and pheasant nests on four 36 square mile blocks of land in northeastern South Dakota. The Mayfield Method (Mayfield 1961) will be used to calculate nest success. Two blocks will be trapped for predators and results will be compared between the trapped and the two untrapped blocks. Hypothesizing that block predator management will significantly increase nesting success, it will be one of our ultimate goals to determine if this will be a viable and cost effective management option for managers in South Dakota.
EXPRESSION OF VARIOUS SURFACE MARKERS ON THE PORCINE AND HUMAN INTESTINAL EPITHELIAL CELL LINES.

YeJin Oh
Department of Biology & Microbiology

Gopakumar Moorkanat
Department of Biology & Microbiology
Center for Infectious Disease Research & Vaccinology

Radhey S. Kaushik
Department of Biology & Microbiology
Center for Infectious Disease Research & Vaccinology
Department of Veterinary Science

South Dakota State University
Brookings, SD 57007

ABSTRACT

Intestinal epithelial cells play an important role in mediating innate immune responses and influence the development of adaptive immunity in the gut. Recently, intestinal epithelial cells have been shown to express MHC class I, class II and CD1 molecules consistent with their ability to present antigens. These cells also express many other surface markers involved in various immunological processes. Pigs are frequently used as an animal model for many enteric human diseases. Porcine intestinal cells have been shown to be involved in the pathogenesis and immunity to many porcine enteric diseases. Two porcine intestinal epithelial cell lines IPEC-J2 and IPEC-1 derived from jejunum and small intestine respectively, have been frequently used in many studies but these cells have not been characterized for their surface markers. This study assessed the expression of CD1, MHC-I, MHC-II, CD11b/c, CD14, CD18, wCD21, CD25, CD40, CD44, CD54, CD58, CD80, CD86, and CD172a on porcine IPEC-J2 and IPEC-1 cells and human small intestinal cell line INT-407 by flow cytometry. A variable number of both human and porcine cells expressed MHC-I, MHC-II, and CD44. Both IPEC-J2 and IPEC-1 cells expressed CD11b/c but did not express CD54 and CD58; however, INT-407 cells expressed both CD54 and CD58 but no CD11b/c. None of the porcine and human cell lines expressed CD1, CD14, CD18, wCD21, CD25, CD40, CD80, CD86, and CD172a. These findings suggest that both IPEC-J2 and IPEC-1 cells may act as possible antigen presenting cells and provide suitable porcine cell culture models for studying the intestinal immune mechanisms.
THE EVOLUTION OF TEACHING:
COMPARING THE TEACHING OF THE THEORY
OF EVOLUTION IN SOUTH DAKOTA PUBLIC
HIGH SCHOOLS OVER THE LAST 20 YEARS

Carol M.F. Wake and Laura M. Wake
Department of Agriculture and Biology
South Dakota State University
Brookings, SD 57007

ABSTRACT

Understanding the theory of evolution, which lies at the heart of all sciences, is crucial for public high school students. Many surveys have been conducted throughout the U.S. to track the level of understanding and instruction of biological evolution among educators. Our study focuses primarily on South Dakota teachers. We sent questionnaires to a cross-section of SD high school biology teachers to ascertain their understanding and acceptance of the scientific method and their teaching strategies regarding the theory of evolution. The questions focused on quantitative information regarding science class curriculum to qualitative information including subjective interpretation of community or administrative pressures to teach only certain science subjects. Similar surveys were conducted in 1988 and again in 1999. We compared the results of this survey with past survey results and our data indicated how teaching has changed over the last 20 years in SD. To date there remains low understanding and teaching of the theory of evolution in classrooms. A correlation is indicated between an educator’s understanding and acceptance of the scientific method and biological evolution and the level of student knowledge regarding the theory of evolution.
UNDERSTANDING N MASS BALANCE IN A LONG-TERM PRODUCTION FIELDS USING VARIOUS TOOLS

K. Kim, D.E. Clay, C.G. Carlson and S.A. Clay
Plant Science Department
South Dakota State University
Brookings, SD 57007

ABSTRACT

Monitoring long-term impacts of fertilizer N and the fate of N sources in production fields are needed. The objective of this study was to determine total soil N temporal and spatial changes over an 8 year period in a ¼ section. The site was located in east-central South Dakota in a 65-ha field. Crop rotation was corn and soybean. During the study corn was grown for 5 years (1995, 1997, 1999, 2001, and 2002) and soybeans were grown for 3 years (1996, 1998, and 2000). Two tillage methods were used; no-till between 1995 and 1999 and strip-till between 2000 and 2002. More than 600 soil samples from the 0- to 15-cm soil depth were collected from a 30- by 30-m offset grid in May, 1995 and between May and June, 2003 and were aggregated to a common 40- by 40-m grid cell. Soil samples were air dried (35 °C), ground, sieved, and analyzed for total N, total C, $\delta^{13}$C discrimination ($\Delta$), and $\delta^{15}$N on a ratio mass spectrometer. Findings from this study can be used to an improved understanding of N cycling in production fields.
ABSTRACT

Quality is very important in the production of wheat. Differences in the characteristics of wheat flour determines its use, be it bread, pasta, and so forth. These differences also reflect a difference in price per bushel. Cultural practices and climate can have a large effect on the quality of wheat. These factors affect many different aspects of the wheat industry from the plants in the field to their final products in the market. In this study different varieties of winter and spring wheat, geographical location, and historical wheat data are taken into consideration. Data collection is primarily done through soil and grain samples, as well as, weather stations. Also, selenium levels in the floor and soil has been analyzed, a common attribute in South Dakota. Analysis through Geographic Information Systems (GIS) is the primary tool used for data analysis and display. This project is supported from the South Dakota Wheat Commission.

Keywords

Wheat, flour, selenium, climate, GIS
DETERMINATION OF THE RATIO OF TOTAL THIOLS AND DISULFIDES—AN INDEX OF INTRACELLULAR OXIDATIVE STRESS

Wei Chen, Teresa Seefeldt, Yong Zhao, Sarah Hanson, Ryan Foll and Xiangming Guan
Department of Pharmaceutical Sciences
College of Pharmacy
South Dakota State University
Brookings, SD 57007

ABSTRACT

Thiols (-SH) play an important role in maintaining an intracellular reducing environment that is required for normal cellular functions. In addition, thiols also directly detoxify oxidants or act as coenzymes in reactions that detoxify oxidants. Intracellular thiols primarily consist of glutathione (GSH), cysteine (CSH), and protein thiols (PSH). Thiols can be oxidized to disulfides (-S-S-) under various oxidative stress conditions. The ratio of thiols over disulfides has been used as an index of oxidative stress. A decrease in the ratio is related to an increase in oxidative stress. An increase in intracellular oxidative stress has been linked to cell malfunction and various disease states. Therefore, analytical methods to determine total thiols and total disulfides will be useful tools in research related to cellular oxidative stress.

This study describes a modified methodology by which the concentration of the total cellular thiols, as well as their disulfides can be reliably measured. In this study, 5, 5’-dithiobis(2-nitrobenzoic) acid (DTNB) was used as a thiol specific reagent. Thiols reacted with DTNB to yield 2-nitro-5-thiobenzoic acid which was measured spectrophotometrically at 412 nm. Disulfides were quantified after being reduced by sodium borohydride (NaBH₄) to thiols. This method was validated by determining intracellular oxidative stress created by inhibitors of glutathione reductase and glutathione biosynthesis in a human ovarian cancer cell line and monkey kidney cell line. The experimental procedures and results will be presented. [Supported by the National Institutes of Health (CA098810-01, CA120062-01), 2005 Governor Rounds’ Individual Research Seed Grant Award]
INCREASE IN OXIDATIVE STRESS VIA GLUTATHIONE MODULATION AS A NOVEL APPROACH TO ENHANCE CANCER SENSITIVITY TO RADIOTHERAPY

Yong Zhao, Teresa Seefeldt, Wei Chen, Laura Carlson, Adam Stoebner, Sarah Hanson, Ryan Foll, Srinath Palakurthi and Xiangming Guan

Department of Pharmaceutical Sciences
College of Pharmacy
South Dakota State University
Brookings, SD 57007

ABSTRACT

Radiotherapy is one of the major therapies in cancer treatment. The main obstacle in treating cancer by radiation is the resistance of cancer to this therapy leading to treatment failure. Therefore, developing novel approaches to reverse cancer resistance or to increase cancer sensitivity is an ongoing research effort. This project investigates whether increased oxidative stress via glutathione (GSH) modulation can increase cancer sensitivity to radiation.

In this study, OVCAR-3 cells, a human ovarian cancer cell line, was employed for the investigation. Intracellular oxidative stress was created through the inhibition of glutathione reductase (GR) or a combined inhibition of GR and GSH biosynthesis. Inhibition of GR was achieved by G0026, an irreversible GR inhibitor developed in this laboratory. Inhibition of GSH biosynthesis was achieved by buthionine sulfoximine (BSO), an inhibitor of \( \gamma \)-glutamylcysteine synthetase (GCS), the enzyme that catalyzes the first and rate-determining step of GSH biosynthesis. OVCAR-3 cells were plated in a 96 well plate and treated with different drug treatments. Cell viability was determined by the MTT assay. Drug effects were determined by survival rates which were obtained by comparing the cell viability of a drug treatment against that of a control where cells were treated with no drug. Our results show that G0026 at 50 \( \mu \)M, a concentration producing 95% GR inhibition, yielded a significant increase in the sensitivity of OVCAR-3 cells to radiation. A more profound increase in sensitivity was achieved by the combined inhibition. The experimental procedure and results will be presented. [Supported by the National Institutes of Health (CA098810-01, CA120062-01), 2005 Governor Rounds’ Individual Research Seed Grant Award]
IMPROVING THE FERMENTATION CHARACTERISTICS OF CORN THROUGH AGRONOMIC AND PROCESSING PRACTICES

G. Reicks, H. Woodard and A. Bly
Plant Science Department
South Dakota State University
Brookings, SD 57007

ABSTRACT

Four N fertilizer rates were applied to six corn hybrids to determine the effects of agronomic practices on ethanol production from corn grain. Grain was also harvested at two moisture levels and artificially dried at four temperatures to determine the impact of processing factors on ethanol production. Typical N fertilizer rates applied in South Dakota did not decrease ethanol production by increasing grain protein levels. One corn hybrid produced 0.4% more ethanol than two other hybrids. Ethanol production decreased by 0.3% when immature kernels were less than one-half hard starch. Grain moisture level at harvest generally did not affect ethanol production. The optimal temperature for artificially drying corn grain was between 100 and 125°F. Ethanol production was decreased by 0.1 to 0.3% when drying grain at 75°F due to microbial deterioration. Ethanol production was decreased by 0.1 to 0.4% when drying grain at 140 to 200°F due to starch bonding with protein. A multiple regression model was developed to predict ethanol production based on grain density and near infrared transmittance (NIT) analysis of grain samples for starch, protein, oil, and extractable starch concentrations. These variables were inadequate predictors of ethanol production ($r^2=0.37$). The amount of grain starch converted into glucose, the energy source for ethanol-producing yeasts, also did not effectively predict ethanol production ($r^2=-0.07$). These findings suggest that farmers can contribute to increased ethanol production and that factors to predict ethanol production still remain unknown.
THE MOVEMENT OF ANTIMICROBIAL CHEMICALS IN SOIL

Aaron Hoese, Susan Gibson, Volker Brozel
Biology Department

S. A. Clay
Plant Science Department

South Dakota State University
Brookings, SD 57007

ABSTRACT

Tylosin (Tyl) and chlortetracycline (CTC) are antimicrobial chemicals used as growth promoters in cattle, swine, and poultry production and can be excreted as the parent compound. Landspreading manure can move these chemicals into the soil. The objective of this study was to determine how Tyl and CTC from pig slurry applied to soil moved during simulated rainfall. Three application sites in Brookings County were used. The first year slurry with high concentrations of CTC and Tyl (118 mg and 0.110 mg respectively) was used. The second year slurry with low concentrations of CTC and Tyl (14.3 mg CTC; Tyl was only detected not quantifiable) was used. Rainfall was simulated using Cornell Sprinkler Infiltrometers. The high concentration year had percent recovery for CTC ranging from 0.86 to 3.54% for CTC and 8.40 to 12.07% for Tyl from rainfall runoff. The low concentration year had no recovery from rainfall runoff. Soil extractions yielded no antibiotics either year.
NOVEL ALTERNATIVE SPLICING AND EXON SHUFFLING OF A TOMATO CALMODULIN GENE VARIANT

Daniel Bergey and Anna Hermanson
Black Hill State University
Spearfish, SD

ABSTRACT

Calmodulin (CaM) is a cytosolic, calcium binding protein that “senses” intracellular fluctuations of free Ca++ and transduces the calcium second messenger by initiating appropriate cellular responses, including the activation of individual enzymes or specific biochemical cascades. We have cloned and sequenced the entire tomato calmodulin gene family, consisting of 6 distinct genes, and further determined that one of these genes is alternatively spliced to produce two different mRNA products, each encoding a different CaM isoform. One the CaM isoforms is a standard cytosolic calmodulin protein; whereas the other isoform is a modified calmodulin containing a C-terminal, 25 amino acid long nuclear localization signal (NLS) encoded by a separate exon. Sequence database searches revealed an unpublished calmodulin cDNA entry from Petunia (an ancestral Solanaceae family member) containing a homologous C-terminal NLS-domain. We are using this experimental system to investigate molecular processes that drive the evolution of “new” genes, and the role of alternative splicing regulating gene expression.
Special Symposium:
New Paradigms in Signal Transduction
presented at
The 92nd Annual Meeting
of the
South Dakota Academy of Science
Background: Dystrophin associates with a protein complex (DAPC) which is composed of: The extracellular protein, α-dystroglycan; The transmembrane proteins, β-dystroglycan and α, β, γ, δ - sarcoglycans; and caveolin-3. A δ-sarcoglycan deficiency manifests as Limb Girdle Muscular Dystrophy 2F, with dilated cardiomyopathy (DCM) occurring in patients. A hamster strain that is δ-sarcoglycan deficient (TO-2 DCM) was identified as a DCM model. Our studies demonstrate an enhancement of ERK and Akt activation in the TO-2 DCM model.

Methods: Kinase activation and substrate phosphorylation was determined by immuno-blotting with phospho-specific antibodies for ERK and Akt pathways.

Results: Enhanced MEK1 and ERK activation was observed in the TO-2-DCM model as compared to the F1B control animal. ERK activation in these models was accompanied by an increase in Raf Ser259 phosphorylation. This was unexpected in that this Akt mediated phosphorylation event reportedly inhibits the ERK pathway. Additionally, enhanced phosphorylation of the ERK and Akt substrates: Elk-1 and GSK-3 was observed in the TO-2 DCM group. Finally, enhanced phosphorylation of Akt Tyr326 was observed in the TO-2 DCM group. Activation of these signal transduction pathways was accompanied by myocardial dissociation of the extracellular protein- α-dystroglycan.

Conclusion: We propose that the loss of α-dystroglycan dissociates the linkage of cardiomyocytes to the extracellular matrix protein-laminin. This dissociation leads to a destabilization of the myocardial sarcolemmal membrane, activating a series of signal transduction pathway in response to this structural breakdown. This corresponds to studies which demonstrate enhanced ERK activation and cardiomyopathy in a caveolin-3 knockout mouse model.
CELL SIGNALING THROUGH MUCINS IN CANCER

Subhash C. Chauhan and Meena Jaggi
Cancer Biology Research Institute
Sanford Research/The University of South Dakota
Sioux Falls, SD 57105

ABSTRACT

The members of mucins family are heavily glycosylated high molecular weight proteins which are primarily involved in the protection and lubrication of luminal epithelial surfaces. Mucins are also known to be aberrantly expressed in cancer cells. Their role as signaling molecules, however, has emerged only in past decade. The transmembrane mucins are involved in signal transduction, through extracellular domain-mediated ligand binding or by interacting with growth factors receptors. Additionally, the cytoplasmic tail of certain mucins such as MUC1 is involved in several signaling pathways, including Ras, beta-catenin, p53 and estrogen receptor pathways. Recently, MUC1 expression was found to be localized on mitochondrial membranes that initiated a debate if mucins can alter the apoptotic pathway in cancer cells. The signaling pathways those are modulated by mucins and their possible role in cancer cells will be presented and discussed during the presentation.
EFFECT OF PROTEIN KINASE D FAMILY MEMBERS ON β-CATENIN/T CELL ACTIVITY IN COLON CANCER CELLS

J. E. Hughes, K. Dunham, S. C. Chauhan, and M. Jaggi
Cancer Biology Research Center
Sanford Research/USD
Department of OBGYN and Basic Biomedical Science
Sanford School of Medicine
University of South Dakota

ABSTRACT

β-catenin is involved in cell adhesion, signal transduction, cellular proliferation, and differentiation. In many colon cancers, β-catenin is not degraded properly due to a mutation in the Adenomatous Poli Coli (APC) gene. This leads to β-catenin acting as a co-transcription factor by in the nucleus pushing the cell into a highly proliferating and non-differentiating stage of growth. Protein Kinase D family members (PKD1, 2, 3) are a family of serine kinases involved in modulating many signal transduction pathways. We analyzed the effect of PKD1, 2, and 3 on β-catenin transcription activity in a colon cancer cell line (SW480) with APC mutation. PKD1, 2, 3, and β-catenin were first localized in the SW480 cells by using specific antibodies and confocal microscopy. SW480 cells were then transfected with either an isoform of PKD or just the vector; TOP or FOP, and pRL-tk DNA constructs. Through the use of a dual reporter assay kit we were able to examine the effect of the PKD family members on β-catenin transcription activity. Most notably our results showed that exogenous expression of PKD1 or PKD2 decrease β-catenin/TCF activity. Through an immunohistochemical study down regulation of PKD1 staining was found in colon cancer samples that were moderately and poorly differentiated and had a higher Dukes stage as compared to lower Duke stage colon samples. These results point to a novel regulation pathway in normal cells and point to a possible area of deregulation in colon cancer cells.
MODULATION OF $\beta$-CATENIN SIGNALING BY PROTEIN KINASE D1 IN PROSTATE CANCER CELLS

Katrina Dunham, Joshua E. Hughes, Subhash C. Chauhan and Meena Jaggi
Cancer Biology Research Center
Sanford Research/USD
Department of OBGYN and Basic Biomedical Science
Sanford School of Medicine
The University of South Dakota
Sioux Falls, SD

ABSTRACT

Protein Kinas D1 (PKD1) is a serine kinase involved in modulation of several signal transduction pathways in benign and malignant human diseases. $\beta$-catenin is a cell adhesion molecule involved in signal transduction and cellular proliferation and differentiation. We examined the effect of PKD1 on $\beta$-catenin/T cell factor (TCF) transcription activity using transiently transfected reporter constructs (TCF and pRL-TK constructs) and cell cycle progression using flow Cytometry in prostate cancer C4-2 cells stably transfected with green fluorescent protein (GFP) or PKD1 fused GFP. The luciferase activities were assayed using Dual Glo reporter assay with a luminometer. Specificity of PKD1 mediated alteration of subcellular distribution of $\beta$-catenin was confirmed by silencing PKD1 expression using small interfering RNA (RNAi) in C4-2-GFP-PKD1 cells. Increased PKD1 expression in C4-2-GFP-PKD1 cells lead to two-fold reduction in $\beta$-catenin mediated transcriptional activity while decreased PKD1 expression by RNAi resulted in a significant increase in $\beta$-catenin mediated transcription activity. C4-2-GFP and C4-2-GFP-PKD1 cells were labeled using Telford method for flow cytometry measurement. As a consequence of PKD1 expression, the percentage of cells in the S and G2/M phases of the cell cycle declined, whereas the number of cells arrested in G1 phase increased.
ROLE OF MUCOSAL IgG RESPONSE IN PROTECTION AGAINST AVIAN INFECTIOUS BRONCHITIS VIRUS

Xueshui Guo and Xiuqing Wang
Department of Biology and Microbiology

Din Chen
Department of Mathematics and Statistics
South Dakota State University
Brookings, SD 57007

Artur Rosa
Department S.En.Fi.Mi.Zo.- Animal production Section
University of Palermo-Viale delle Scienze-Parco d’Orleans
90128 Palermo, Italy

ABSTRACT

Avian infectious bronchitis virus (IBV) infection is one of the major viral respiratory diseases of chicken and causes significant economic losses to the poultry industry worldwide. The timely development of a potent, mucosal immune response plays a pivotal role in protection against IBV infection. In the previous study, we have reported that a variety of innate immunity and a Th1 based adaptive immunity were activated in the host’s early defense (3 days after the initial exposure to IBV) against the first IBV exposure and they are responsible for the rapid clearance of virus from the local infection. To understand the molecular basis of immune protection against the second IBV exposure, we examined the local immune related gene transcription profiles at 1 and 3 days after the second exposure to IBV using 13k DNA microarray. Results suggested that the expression of Ig gamma chain was up regulated significantly in local mucosal tissues after the second exposure of IBV. In contrast, T cell immunoglobulin mucin-3, T cell receptor zeta chain subunit, T cell signal transduction molecule SAP, granzyme A precursor, and Fas antigen, which were all significantly up regulated after the first exposure, did not show obvious increase in expression following the second virus exposure, suggesting that mucosal IgG immune response, rather than T cell immunity, was critical in the prevention of virus entry during the second exposure to IBV. Due to the lack of Ig alpha chain in the 13k microarray, we could not directly assess the role of mucosal IgA in protection against IBV. However, data obtained from real-time RT-PCR suggested that the expression of Ig alpha chain transcript was suppressed. This was further supported by the down-regulated expression of Ig J chain (linkage component of IgA) and transforming growth factor beta (the gene regulator for IgA). Overall, our data suggested that mucosal IgG, rather than T cell immunity and IgA, might be critical in protection against secondary IBV exposure locally.
REVIEW OF CURCUMIN EFFECTS ON SIGNALING PATHWAYS IN CANCER

Bal Krishan Jaggi, Subhash C. Chauhan and Meena Jaggi
Sanford Research/USD, Cancer Biology Research Center
Obstetrics and Gynecology and Basic Biomedical Sciences Departments
Sanford School of Medicine of The University of South Dakota and Sanford Health
Sioux Falls, SD 57105

ABSTRACT

Cancer is a major health problem in the United States. In recent years, the use of natural dietary supplements for cancer prevention and treatment has received considerable attention. One such natural dietary supplement is curcumin, a major constituent of the herb called turmeric, a common spice used in Indian food. Turmeric is the dried ground rhizome of the perennial herb Curcuma longa Linn of the ginger family. Extensive studies relating to its potential role in cancer prevention and treatment suggest that curcumin consumption may reduce the risk of cancer. Curcumin prevents cancer development and progression by interfering with several cell signaling pathways which are commonly associated with cancer cell proliferation and metastasis. Its intervention in NF-κB, MAPK, Akt, p53 and steroid receptor pathways has already been established, while its involvement in other pathways is still under investigation. Curcumin also has potent radio-sensitizing activity, and its use in combination with radiation based therapy may provide a better treatment outcome in cancer patients. The herbal product curcumin is now emerging as a new hope in the fight against cancer.

Key words

Cancer, curcumin, cancer prevention, herbs for cancer, signaling pathways

INTRODUCTION

Curcumin is a major active constituent of turmeric, which is a well-known herb in the ancient Indian System of Medicine “Ayurveda” (Nadkarni and Nadkarni 1976). Turmeric is the dried ground rhizome of the perennial herb Curcuma longa Linn of the ginger family. It is called turmeric in English, Haldi or Haridra in Hindi, and Ukon in Japanese. The color of turmeric powder is orange yellow and it tastes a little sour. The role of some of these natural dietary supplements on human health is the subject of scientific investigation. A review of the literature revealed that turmeric is useful in treating a variety of ailments and metabolic disorders (Khanna 1999). More than two hundred and fifty research papers relating to curcumin were published in the past year according to a search
of the U.S. National Library of Medicine. The U.S. National Institutes of Health has four clinical trials underway to study curcumin for treatment of pancreatic cancer, multiple myeloma, colorectal cancer and Alzheimer’s disease. In this article, we specifically describe the effects of curcumin on signaling pathways that highlights some vital roles of curcumin in the treatment of cancer.

BIOLOGICAL PROPERTIES

Turmeric has drawn world-wide attention for its medicinal properties originating back to 1815 (Vogel and Pelletier 1815). It was first chemically characterized in 1910 (Lampe, V. et al. 1910). The active constituent of turmeric is curcumin, also known as C.I. 75300 or Natural Yellow 3, (1E, 6E)-1, 7-bis (4 hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione. It can exist in two tautomeric forms, keto and enol. The keto form is preferred in solid phase and the enol form in solution (Figure 1). Curcumin and its metabolites, mainly tetrahydrocurcumin, hexahydrocurcumin, glucuronides (Holder, Plummer et al. 1978), and essential oil, isolated from turmeric rhizome, are being investigated for the prevention and treatment of cancer. Curcumin is a natural, nontoxic food constituent. On irradiation with visible light, curcumin proves to be phototoxic for Salmonella typhimurium and Escherichia coli, even at very low concentrations. The observed phototoxicity makes curcumin a potential photosensitizing drug which might find application in the phototherapy of psoriasis, cancer, bacterial and viral diseases (Tønnesen, de Vries et al. 1987).

Extensive research in the last 30 years has indicated that curcumin has both preventive and therapeutic abilities for cancer. Beside antioxidant, anti-inflammatory, cancer chemo-preventative, and potential chemotherapeutic properties,
curcumin also protects against various forms of stress, cataract formation, alcohol-induced liver injury, drug-induced myocardial toxicity, bowel diseases, lung injury, nephrotoxicity and stroke. Multiple biological activities of curcumin are shown in Figure 1.

CURCUMIN AND CANCER

Cancer is a major health problem in the United States. It is not just one disease, but a complex family of diseases. The term cancer actually encompasses more than 100 diseases that affect many different tissues and cell types. All cancers start from normal cells that have gone awry. Cancer is simply an abnormal tissue made of abnormal cells. In 2000, Douglas Hanahan and Robert Weinberg (Hanahan and Weinberg 2000) described six major characteristics of cancer cells, i.e. self-sufficiency in growth signals, insensitivity to anti-growth signals, evading apoptosis, limitless replicating potential, sustained angiogenesis and tissue invasion and metastasis. Various studies on curcumin have shown promising results in effectively intervening and/or controlling most of these hallmarks of cancer progression as shown in Figure 2. Curcumin, with its chemo-preventative and anti-carcinogenic properties, offers hope for the care and cure of several types of cancers, such as gastric, colon, stomach, liver, lung, duodenum, oral (Tanaka, Makita et al. 1994), skin (Conney, Lysz et al. 1991) breast (Wang and Wieder 2004), cervical, and prostate cancers (Lin, Shi et al. 2006). Several studies indicate that curcumin slows the development and growth of numerous types of cancer cells. Topical application of curcumin has also been shown to inhibit chemical carcinogenesis of the skin by Conney et al (Conney, Lysz et al.

![Figure 2. Schematic representation of curcumin modulated signaling pathways that are involved in cancer pathogenesis and progression. Green arrows represent activation and red arrows represent inhibition of the pathway. (AR=Androgen Receptor, ER=Estrogen Receptor, MMPs= Matrix Metalloproteinases)
Curcumin affects diverse cellular processes of cancer cells. An ethanol extract of turmeric, "Curcuma longa," as well as an ointment of curcumin (its active ingredient) were found to produce remarkable symptomatic relief in patients with external cancerous lesions. Reductions in smell and itching were noted in 90% and 100% of the cases respectively (Kuttan, Sudheeran et al. 1987). Dry lesions were observed in 70% of the cases, and a small number of patients (10%) had a reduction in lesion size and pain. In many patients the effect continued for several months. An adverse reaction was noticed in only one of the 62 patients evaluated (Kuttan, Sudheeran et al. 1987).

MECHANISM OF ACTION

In vivo and in vitro studies have demonstrated curcumin's ability to inhibit carcinogenesis at three stages: tumor promotion, angiogenesis, and tumor growth. The molecular basis of the anti-carcinogenic and chemo-preventative effects of curcumin are attributed to its effect on several targets, including transcription factors, growth regulators, adhesion molecules, apoptotic genes, angiogenesis regulators cellular signaling molecules and androgen responsive genes (Duvoix, Moreau et al. 2003a; Duvoix, Moreau et al. 2003a; Duvoix, Blasius et al. 2005) (Figure 2). The ability of curcumin to induce apoptosis in cancer cells without cytotoxic effects on healthy cells contributes to the understanding of the anti-cancer potential of curcumin (Sarkar and Li 2004). However, the precise molecular mechanisms of its actions are not fully known. In order to determine curcumin efficacy and its precise mechanism of action, we must understand its molecular interactions and the intracellular signaling network involved in the process.

Curcumin and Nuclear Factor-kappaB (NF-kB) Pathway

Various studies have shown active involvement of the NF-kB pathway in signaling that is employed in controlling cancer cell growth, apoptosis, inflammation and stress response (Shishodia, Amin et al. 2005). There are several important molecules such as NF-kB, IkB, and IKK, which are known to be involved in NF-kB pathways. However, among all these protein molecules, NF-kB is the most important molecule in this pathway and has emerged as a major therapeutic target in cancer cells in recent years. It has been reported in a number of studies that curcumin restrained the expression of IKK, suppressed both constitutive and inducible NF-kB phosphorylation/activation and potentiated TNF-induced apoptosis (Bharti, Donato et al. 2003). Additionally, curcumin showed antioxidant and anti-cancer properties via regulation of the expression of genes required for activation of activator protein 1 (AP1) and NF-kB (Aggarwal, Kumar et al. 2003).
Effect of Curcumin on β-Catenin Signaling

Inappropriate activation of the β-catenin signaling pathway is linked to a wide range of cancers, including colorectal cancer, prostate cancer and melanoma. Abnormalities in the expression and functional activity of the E-cadherin/β-catenin complex is implicated in the development and progression of the majority of colon cancers by regulating cell polarity, differentiation, proliferation, migration and invasion (Wheelock and Johnson 2003), (Yap 1998). Curcumin prevents tumors in C57BL/6J-Min/+ (Min/+) mice by decreased expression of the oncoprotein β-catenin with increased enterocyte apoptosis and proliferation in the enterocytes of the Min/+ mouse. These animals bear a germline mutation in the Apc gene and spontaneously develop numerous intestinal adenomas by 15 weeks of age. At a dietary level of 0.15%, curcumin decreased tumor formation in Min/+ mice by 63%. Jaiswal et al. (Jaiswal, Marlow et al. 2002) suggested that curcumin down-regulates β-catenin’s transcriptional activity in HCT116 intestine cancer cells inducing G2/M arrest. Curcumin and its analog, CHC007, are good inhibitors of the β-catenin/TCF signaling pathway in kidney and colon cancer cells.

Protein Kinase D (PKD) family members are important modulators of several signal-transduction pathways in benign and malignant human diseases (Jaggi, Rao et al. 2003; Jaggi M. 2006). We have identified a novel interaction between E-cadherin/β-catenin and PKD1 (Jaggi, Rao et al. 2005). Studies from our laboratory have shown that the β-catenin is phosphorylated by PKD1 and that over-expression of PKD1 in cancerous cells leads to decreased β-catenin/TCF transcriptional activity (Jaggi and Balaji 2006). We are studying the effect of curcumin on the interaction between PKD1 and E-cadherin/β-catenin complex and on β-catenin transcription activity in colon, prostate and cervical cancer. An understanding of the effect of curcumin on the β-catenin signaling pathway will establish a mechanistic basis by which cell adhesion and proliferation can be manipulated in cancer cells.

Effect of Curcumin on p53 Pathway

The p53 tumor suppressor and transcription factor is a vital regulator in many cellular processes including apoptosis, cell cycle control, cellular response to DNA-damage, genomic stability and signal transduction (Liu and Seidel-Dugan 2006). Aberrant expression of p53 is thought to be a hallmark of cancer cells. Its expression status is an important mediator in the cellular response to cancer therapeutic agents. It has been observed that curcumin induces apoptosis at G(2) phase of the cell cycle mammary epithelial carcinoma cells in which cyclin D1 is deregulated but does not affect mammary epithelial cells. Curcumin selectively increases p53 expression at G(2) phase in cancer cells and leads to cytochrome release from mitochondria, an essential requirement for apoptosis (Choudhuri, Pal et al. 2005). In experiments using p53-null as well as dominant-negative and wild-type p53-transfected cells, it has been established that curcumin induces apoptosis in carcinoma cells via a p53-dependent pathway (Choudhuri, Pal et al. 2005).
Mitogen Activated Protein Kinase (MAPK) Pathway and Curcumin Intervention:

The MAPK pathway has also been considered to be a potential target for cancer therapy and prevention. It has been reported that the MAPK pathway consists of a three step kinase system where a MAPKKK activates a MAPKK which activates a MAPK (ERK, JNK, and p38). This pathway results in the activation of cell growth, cell survival and the NF-κB pathway. Curcumin is known to modulate the MAPK signaling pathway and this might contribute to the inhibition of inflammation. Curcumin is able to attenuate experimental colitis through a reduction in p38 MAPK activity. Furthermore, curcumin has a strong repression of the PMA-induced phosphorylation of ERK, JNK, and p38 MAP kinases.

Curcumin and AKT Pathway

The Akt pathway plays a critical role in cell survival and cell growth. This pathway has been shown to be activated in various cancers. Akt is activated by phospholipid binding and phosphorylation at Thr308 by PDK1 and at Ser473 by PDK2. Activated Akt is known to promote cell survival by inhibiting apoptosis via inactivation of several pro-apoptotic factors including Bad, caspase-9 and Forkhead transcription factors. Recent studies have also shown that the NF-κB pathway is regulated by Akt via modulation of phosphorylation and activation of molecules involved in the NF-κB pathway. Therefore, Akt has also been considered to be an attractive target for cancer treatment and prevention. Studies have shown that the Akt signaling pathway is constitutively activated in human T-cell leukemia virus type I (HTLV-I)-infected T-cell lines and in primary adult T-cell leukemia (ATL) cells (Tomita, Matsuda et al. 2006). The effect of curcumin was investigated on Akt activity in HTLV-I-infected T-cell lines and primary ATL cells (Tomita, Kawakami et al. 2006). In these experimental studies curcumin was shown to decrease phosphorylation of PDK1 and thereby inhibit constitutive activation of Akt. Curcumin activated glycogen synthase kinase (GSK)-3beta, a downstream target of Akt kinase, by inhibiting phosphorylation of this protein. Curcumin reduced the expression of cell cycle regulators cyclin D1 and c-Myc proteins, which are both degraded by activated GSK-3beta. Thus, curcumin may have anti-cancer properties which are mediated, at least in part, by inhibiting Akt activity (Tomita, Kawakami et al. 2006).

Effect of Curcumin on Steroid Receptor Pathway

Curcumin has been found to regulate the molecules involved in the androgen receptor (AR) signaling pathway in cancer cells (Guo, Yu et al. 2006). The effects of curcumin on cell growth, activation of signal transduction, and transforming activities in both androgen-dependent and -independent cell lines have been evaluated. Curcumin down-regulates transactivation and expression of AR and AR-related cofactors (AP-1 and NF-κB), and reduces the ability to form colonies in soft agar. The results showed that some curcumin analogues possessed
potent anti-androgenic activities and were superior to hydroxyflutamide, which is the currently available anti-androgen used for the treatment of prostate cancer (Nakamura, Yasunaga et al. 2002; Yang, Zhang et al. 2005).

Experimental evidence suggests that curcumin exerts multiple different suppressive effects on human breast carcinoma cells in vitro. In ER-positive MCF-7 cells, curcumin treatment showed an effective suppression of the downstream genes of the ER pathway, including pS2 and TGF-beta (transforming growth factor). In addition, curcumin exerts strong anti-invasive effects in the ER-negative MDA-MB-231 breast cancer cells (Shao, Shen et al. 2002). These anti-invasive effects appear to be mediated through the down-regulation of MMP-2 (matrix metalloproteinase) and the up-regulation of tissue inhibitor metalloproteinase-1 and 2 (TIMP-1 and TIMP-2). These are molecules that have often been implicated in regulating tumor cell invasion.

CURCUMIN MODULATES RADIO-SENSITIVITY IN CANCER CELLS

Curcumin shows a growth inhibitory effect in a broad range of cancers as well as in TPA-induced skin cancers in animal models. The effect of curcumin has been investigated in radiosensitization in the p53 mutated prostate cancer cell line PC-3. Curcumin at 2 and 4 µM concentrations in combination with radiation showed significant enhancement of radiation-induced clonogenic inhibition and apoptosis. It has also been reported that curcumin inhibits TNF-alpha-induced NF-κB activity that is essential for Bcl-2 protein induction. In PC-3 prostate cancer cells, radiation induced up-regulation of TNF-alpha and NF-κB activity, and resulted in the induction of Bcl-2 protein. Radiation treatment in combination with curcumin showed a strong inhibition of Bcl-2 protein expression via down-regulation of TNF-alpha-mediated NF-κB activity. In addition, a significant activation of cytochrome c, caspase-9 and caspase-3 were also observed in curcumin combined radiation treatments. However, Bax protein expression remained constant in PC3 cells after radiation and/or curcumin treatments. Effective modulation in the expression of these proteins by curcumin caused the enhanced radiosensitization in these cancer cells. These observations clearly demonstrate that curcumin has a potent radio-sensitizing activity, and its use in combination with radiation based therapy may provide a better treatment outcome in cancer patients.

PHARMACOLOGICALLY SAFE AND EFFECTIVE DOSE OF CURCUMIN

At pre-clinical and clinical levels, curcumin has been found to be safe at 3.6 -10 g per day (Aggarwal, Kumar et al. 2003; Sharma, Euden et al. 2004; Sharma, Gescher et al. 2005). Systemic pre-clinical studies funded by the Prevention Division of the U. S. National Cancer Institute found no adverse effects in rats, dogs or monkeys in doses of up to 3.5 g/kg BW, administered for up to 3 months
A daily oral dose of 3.6 g of curcumin results in pharmacologically effective levels in colorectal tissue with negligible distribution of the parent drug in hepatic tissue or other tissues of the gastrointestinal tract (Garcea, Berry et al. 2005). No toxicity was observed in a study where a high dose of oral curcumin was administered (up to 8 g curcumin daily for 3 months) to patients with pre-invasive malignant or high-risk pre-malignant conditions (Cheng, Hsu et al. 2001).

Due to lack of any substantial data in favor of a dose response relationship for a biomarker of curcumin’s activity, several observations in human volunteers and patients suggest that curcumin may possess systematic biological activity at low oral doses. In a small study, a single oral dose of 20 mg curcumin appeared to induce contraction of the gall bladder, assessed by ultrasound scanning in human volunteers, compared to amyllum placebo (Rasyid, Rahman et al. 2002).

PROSPECTIVE

The data generated from in vitro experiments and in vivo preclinical and clinical trials indicates that curcumin exerts inhibitory effects on carcinogenesis and tumor progression suggesting that it has enormous potential in the prevention, care, and cure of cancer. There are nearly 50 different chemotherapy drugs that are being used for the treatment of cancer. Some of them are complementary and some are not. These anticancer drugs have low efficacy and severe side effects. Curcumin is one of the safest natural products which controls proliferation, apoptosis, angiogenesis and metastasis. In conclusion, curcumin is now emerging as a new hope in the fight against cancer.

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LITERATURE CITED


GENE TARGETING IN CATTLE—A GENETIC ISSUE WITH AN EPIGENETIC FACET

Zhongde Wang
Dept. of Epigenetics and Development
Hematech, INC
A division of Kirin Pharmaceutical
Sioux Falls, SD, 57106

ABSTRACT

Due to the lack of embryonic stem cells from farm animals, gene targeting in these species is a challenge. Particularly, there was no method available for knocking out a transcriptionally silent gene or for performing double targeting to produce homozygotes in these species. By using animal cloning (somatic cell chromatin transfer), we developed a sequential gene targeting system in using primary bovine fibroblast cells to knock out both alleles of a silent gene encoding immunoglobulin-mu (IGHM). Furthermore, we also sequentially knocked out both alleles of the prion gene encoding bovine prion protein (PRNP) in the IGHM targeted cell line and produced doubly homozygous knockout calves. Since such sequential gene targeting strategy requires multiple rounds of animal cloning, adequately reprogramming the epigenome of the targeted cells by the oocytes became an important issue for consideration. This presentation will discuss our sequential gene strategy in bovine and some of the issues involved in epigenetic reprogramming.
HER2/NEU GENE EXPRESSION PRODUCTS EVALUATED WITH SUPERPARAMAGNETIC, GENETICALLY ENGINEERED ANTIBODIES

Marek Malecki
Department of Pharmaceutical Sciences
College of Pharmacy
South Dakota State University
Brookings, SD 57007

ABSTRACT

HER2/neu is an oncogene amplified and overexpressed in ovary and breast cancer cells. In heterodimers, HER2/neu receptors stimulate signal transduction pathways leading to increased cell proliferation. The level of its expression is associated with cancer malignancy. Therefore, HER2/neu is an important indicator of cancer malignancy, as well as, a target for antibody guided cancer therapies. The main objective of this work was to develop molecular probes, which would report levels of HER2/neu gene expression and anatomical distribution of its products in vivo. Magnetic resonance imaging (MRI) is particularly suitable for this endeavor, as it offers not only the best spatial resolution from all in vivo imaging modalities currently available, but also the topographic reference for location of these probes within anatomy of the human body. Superparamagnetic single chain variable fragment (scFv) antibodies targeting HER2/neu were genetically engineered. They warranted high labeling specificity and affinity revealed with EDXSI, as well as, induced significant changes in relaxivity detected with NMR. This study demonstrated a proof of concept for using superparamagnetic scFv in diagnostic evaluation of levels of gene expression products with NMR and MRI for planning receptor targeted therapy.

Keywords

HER2/neu, ovarian cancer, breast cancer, signal transduction, genetically engineered antibodies

INTRODUCTION

Her2/neu is an oncogene amplified and overexpressed in ovarian and breast cancer cells (Di Fiore et al 1988, Berger et al 1988, Guerin et al 1988, van de Vijver et al 1988, Slamon et al 1989, Nielsen et al 2007). The level of its expression is associated with cancer malignancy (Berchuck et al 1990, King et al 1992, Zagouri et al 2007, Robert & Favret 2007). The ovarian or breast cancer cells may have approximately 3x10^6 expressed from multiple copies of the gene, while healthy cells in these organs may have approximately 2x10^5 HER2/neu receptors
on their surfaces. This leads to great increase in stimulations of signal transduction pathways, thus accelerated cell cycles and increased cell proliferation (King et al 1988, Lahusen et al 2007). Her2/neu positive cancers are the most invasive and have the worst prognosis. Therefore, levels of gene expression products and their distribution determined with monoclonal antibodies are of great diagnostic and prognostic value (Harris et al 1989). Furthermore, they are currently the primary target for antibody-guided, receptor-targeted therapies (Hudziak et al 1989, Jorgensen et al 2007, Park et al 2007, Allen et al 2007).

Ex vivo surgical biopsies are the primary material used currently for diagnostic analysis in immuno-histopathology laboratories (Shin et al 2007, Tischkowitz et al 2007, Tuma2007, Carney et al 2007). Many techniques dealing with the evaluation of gene expression and its products include real time qualitative PCR, DNA microarray, differential display, blotting, serial analysis of gene expression (SAGE), etc. Each technique relies upon testing ex vivo of a small tissue or cell sample from a particular anatomical location at the time of biopsy only. However, the cancer gene expression profiles change rapidly, so are the levels and distributions of gene expression products (Fink-Retter et al 2007, Moon et al 2007). Diagnosis and prognosis would be far more accurate, if they would be based upon the images of the entire cancer and projections of its kinetics upon the whole patient’s pathophysiology.

In vivo molecular imaging of these antibodies would greatly facilitate such a diagnosis as well as reduce the patient’s trauma. This could be done with antibody guided contrast in vivo in magnetic resonance imaging (MRI). MRI offers not only the best spatial resolution from all in vivo imaging modalities currently available, but also the topographic reference for location of these probes within anatomy of the human body. The antibody guided probes, could provide the information concerned not only with antigenicity per se, but also report quantitative differences in levels of expression, as well presence of mutations, within architecture of the whole patient’s body at once.

The main objective of this work was to develop antibody guided molecular probes suitable for studying functions and locations of the HER2/neu gene expression products in vivo with MRI.

For developing of new probes for in vivo MRI, it is worth to consider that registered contrast differences between various tissue compartments are generated by local differences in relaxivities of water protons between those compartments. These are translated into varying brightness of the image details on the MRI scanner’s screen. Therefore, it is not as much the strength of the resonance signal itself, but rather the relative differences in signal intensity between various structures and/or in the signal to noise ratios that are the most essential properties in successful visualization of the analyzed features. Gadolinium (Gd) or Europium (Eu) atoms affect water proton relaxivity in their very immediate vicinity. $10^{-5}$ M of Gd is considered to be the threshold for inducing such a change in relaxivity of water, that it will be detected in NMR. If chelated into antibodies, these atoms indirectly report the presence of molecules that were targeted by antibodies. Attempts to realize that idea were conveyed by randomly attaching reporters: Gd chelates, dendrimers, or Fe nanoparticles to monoclonal IgG antibodies, thereby introducing paramagnetic properties (Curtet et al 1985, Mendonca et al 1986,
Linger et al. 1986, Weissleder 1991, Unger et al. 1999, Kobayashi et al. 2003). Two main factors contributed why these attempts did not succeed. Random incorporation of reporters into IgG molecules leads to compromised specificity of antibodies up to their denaturation, thus low specific binding signal and high background due to non-specific binding. Significant increase in size of antibodies due to incorporation of reporters and change of their properties led to steric hindrance and repulsion forces. I have promoted an entirely different approach to improving labeling effectiveness by genetic engineering heterospecific, poly-functional molecules. They were engineered to contain multiple highly specific, separate domains assigned to their functions: scFv guided targeting domain, metal atoms chelating domains, signaling sequences, etc. Upon incorporation of Gd or Eu these molecules were gaining superparamagnetic properties without affecting their targeting functions.

The main hypothesis was, that proportional increase in the number of HER2/neu receptors per cell would result in the proportional increase in Gd atoms anchored via scFv to this cell, and that would result in a proportional increase in relaxivity of the surrounding water leading to the proportional increase in the signal strength recorded with NMR of ex vivo samples or MRI in vivo.

METHODS AND MATERIALS

Cell cultures

The cell lines TOV-112D CRL-11731 and CRL-11732 OV-90 were derived from primary malignant adenocarcinomas of the ovary at grade 3, stage IIIC. They were cultured in a 1:1 mixture of MCDB 105 medium and Medium 199, 85%; donor bovine serum 15% (ATCC). The cells were tumorigenic in nude mice. Cultured in soft agar they formed colonies and spheroids. The cells tested positive for HER2/neu and p53 mutation. The cell line NIH OVCAR-3 HTB-161 was derived from the cells in ascites of a patient with malignant adenocarcinoma of the ovary. The cell line is was grown in RPMI-1640 Medium (ATCC) supplemented with 0.01 mg/ml bovine insulin and donor bovine serum to a final concentration of 20%. The epithelial cells are positive for estrogen and progesterone receptor. They form tumors in nude mice. They form colonies and spheroids grown in soft agars. The cell line CRL-2340 HCC2157 was derived from the ductal carcinoma of the mammary gland tumor classified as TNM stage IIIA, grade 2, with lymph node metastasis. The cells are grown in a 1:1 mixture of Ham’s F12 medium with 2.5 mM L-glutamine and Dulbecco’s Modified Eagle’s Medium adjusted to contain 1.2 g/L sodium bicarbonate with additional supplements (ATCC).

The cell line MCF7 HTB-22. The cells are positive for estrogen receptor and express WNT7B oncogene. The medium to culture this cell line is Eagle’s Minimum Essential Medium (ATCC) with added following components: 0.01 mg/ml bovine insulin; donor bovine serum to a final concentration of 10%. The cell line 184A1 CRL-8798 was originally established from normal mammary tissue and transformed with benzopyrene. The line appears to be immortal,
but is not malignant. The line grows in Mammary Epithelial Growth Medium (MEGM) (Clonetics) supplemented with 0.005 mg/ml transferrin and 1 ng/ml cholera toxin. The normal, adherent fibroblast cell line Detroit 573 CCL-117 was derived from skin. It is grown in Minimum essential medium (Eagle) in Earle’s BSS with non-essential amino acids (ATCC), sodium pyruvate (1 mM) and lactalbumin hydrolysate (0.1%), 90%; fetal bovine serum, 10%. The cells were grown into spheroids within synthetic extracellular matrix.

Superparamagnetic single chain variable fragment (scFv) antibodies

Plasmid constructs were described in the details (Malecki et al. 2002). Coding sequences for variable fragment antibodies (scFvs) targeting HER2/neu selected from the surface displayed libraries were cloned in pM vectors designed with CMV immediate early promoter, SV40 poly(A) termination, hexahistidine, pentaglutamate, and selection neomycin-resistance coding sequences. Constructs for these bi-functional antibodies were electroporated into human myelomas (Malecki 1995; Malecki et al. 2002). Expressions of these constructs resulted in secretion of hetero-specific, poly-functional, mono-valent antibodies. Chelating sites were saturated with metal ions: Gd, Eu. Purification from non-bound metal was performed on affinity columns. The antibodies were produced in modified roller bottles

Freezing and freeze-substitution of cell spheroids

The details of the cryoimmobilization by freezing were described previously and are only briefly presented here. The cells injected into the chambers were rapidly frozen in nitrogen slurry down to down to -196°C. The frozen samples were into methanol precooled to -90°C in the freezer (ThermoNoran). Temperatures were maintained at -90°C, -35°C, and 0°C for 48 hour. Infiltration with Lowicryl preceded polymerization with UV at-35°C and ultramicrotomy. Alternatively, critical point drying was followed by fast atom beam sputter coating.

Immunolabeling

Cell spheroids grown in culture were spun down at 300xg. The cells were resuspended in donor serum to which superparamagnetic antibodies were added. Upon completion of labeling the cells were rinsed with PBS. They were studied with NMR or processed by freezing in preparation for LSCM or EDXSI. Alternatively, cell lysates electrotransferred onto PVDF membranes were immunolabeled with antibodies with or without chelated Gd or Eu atoms.

Determination of metal atoms incorporated into chelating sites

The number of atoms chelated into the metal binding domains of the scFv was determined by using a titration method based on a competition between GdCl₃ and radioactive, carrier-free GdCl₃. Several aliquots of antibody (25 ul)
were incubated for 30 min with 100 ul on GdCl₃ at various concentrations. The same aliquots were incubated for 30 min with 100 uCi of 153 GdCl₃. Free Gd ions were complexed with 10 ul of 0.1 M DTPA. An aliquot of each solution was chromatographed on a silica ITLC (Gelman) support, using 0.1 M sodium citrate, pH5, as eluent. Alternatively, the chelated sites were saturated with Gd. Subsequently, these samples were purified on the gels as outlined above. Finally, they were analyzed with electron energy loss spectral imaging to determine total C to Gd ratio or in other words, the number of Gd atoms per scFv molecule. Alternatively, the scFvs were altered through carboxyl terminal derivatization with 125 I and their chelated sites saturated with 153 Gd. Subsequently, these samples were purified on the gels as outlined above. They were analyzed on a multi-channel analyzer which can display live full-energy spectrum 125 I at energy of 35 keV and 153 Gd at energy of 99 keV and was able to distinguish these two isotopes (Packard Cobra Gamma Counter).

Native electrophoresis

2% agarose gel was poured using a 10 mM Tris, 31 mM NaCl buffer of varying pH, that did not contain any denaturing agents. The samples in their native state were loaded after mixing with glycerol to add density without denaturing the proteins. The gel was run in the same buffer used for pouring the agarose at 60 mAmps until the desired separation was reached. The gel was then stained for 30 minutes in Sypro Tangerine Gel Stain (Invitrogen) diluted in the running buffer before imaging using a FluorImager (Molecular Dynamics).

SDS-PAGE

Electrophoresis was run on 12% polyacrylamide gel. 0.75 thick combs with the 2mm lanes were loaded with standard, cell culture lysates. The samples, after mixing with SDS and DTT containing sample buffers (Sigma) were loaded into the wells. The gels were run using a Tris/Glycine/SDS/DTT running buffers. After the run, the gels were stained with colloidal silver or Sypro Tangerine for imaging using a FluorImager (Molecular Dynamics).

Electrotransfer

After electrophoresis, the samples were immediately onto PVDF. The immunoblot was performed with the Mini Trans-Blot Cell (Bio-Rad) within CAPS: 10 mM 3-[Cyclohexylamino]-1-propanesulfonic acid (CAPS), Tris/glycine transfer buffer 25 mM Tris base, 192 mM glycine, pH 8.3. Prior the transfer the cooling units were stored with deionized water at -20 C. Immediately after electrophoresis the gel, membrane, filter papers and fiber pads were soaked in transfer buffer for 5-10 min. The pre-cooled transfer units were filled with cooled transfer buffer and electrotransfer proceeded at 350 mA.
Laser scanning confocal microscopy

The three-dimensional stacks of the cells labeled with scFv against HER2/neu were imaged with the laser scanning confocal system - Odyssey on the inverted microscope – Olympus. Excitation wavelengths were used: 337, 488, 543, and 588nm. Images were acquired with Kernel filtration and deconvolution of the data was followed by 3D or album display for analysis.

Nuclear magnetic resonance

The wide-bore nuclear magnetic resonance spectrometer operated at 9T (Brucker) with a mouse-cage resonator was used to evaluate relative relaxivity of the samples based upon T1 measurements. T1 spin lattice relaxation time calculated using inversion recovery pulse sequence were measured using inversion recovery imaging with T1= 50-4000 ms in 100 ms increments. T1 was also calculated from T1-weighted fluid-attenuated inversion recovery (T1-FLAIR) sequence (Tr/Te/Flip = 2210/9.6/90), as well as standard T1-weighted imaging sequences (Tr/Te/Flip = 400/6/90).

Energy dispersive x-ray spectral imaging

Supramolecular of the scFv against HER2/neu was performed with Scanning Electron Microscope with Energy Dispersive X-Ray Spectral Imaging System (EDXSI) - Hitachi 3400. Complete elemental spectra were acquired for every pixel of the scans to create the elemental databases. From them, after selecting an element specific energy window, the map of this element atoms’ distribution was calculated with ZAF correction (NIST). As the antibodies were tagged with atoms of Gd, Eu - exogenous elements incorporated into their structure, so was the location of antibodies determined based upon the elemental maps (Malecki 1995, Malecki et al 2002).

RESULTS

The major problem with designing new contrast agents for molecular imaging was lack of methods providing information concerned with their cell surface distribution and subcellular trafficking at the supramolecular level. This situation changed since the introduction of the very sensitive methods of their detection in situ with EELSI and EDXSI (Malecki 1995, Malecki et al 200). There, genetically engineered antibodies tagged with atoms of selected exogenous elements were localized within three-dimensional architecture of cells and cell organelles to determine molecular mechanisms governing their bio-distribution and bio-compatibility. In this study, TOV-112D CRL-11731, OV-90 CRL-11732, CRL-2340 HCC2157, NIH OVCAR-3 HTB-161, MCF7 HTB-22, 184A1 CRL-8798, Detroit 573 CCL-117 cell spheroids were cultured and labeled with anti-HER2/neu superparamagnetic scFv antibodies. In cultured cells were labeled with antibodies chelating Gd or Eu atoms. They were rapidly frozen. Frozen
cells were freeze-substituted with no metal incorporation, infiltrated, and embedded. Distribution of antibodies, harboring metal atoms, in ultrathin sections or cell whole mounts were examined with elemental mapping systems. The antibodies chelating Gd atoms were anchored to the cell surface receptors. Therefore, they were visualized by mapping Gd (Figure 1). That could be only possible due to acquisition of the full spectrum for every pixel of the scan to create the elemental data base. Thereafter, an energy window selected for Gd allowed for extracting element distribution within the entire image to create element distribution map. This elemental map based antibody distribution was projected onto the cell surface ultrastructure to determine localization of superparamagnetic antibodies at the molecular level.

High specificity of superparamagnetic antibodies was also confirmed on Western blots from cell lysates. Exqui-

Figure 1. The ovarian cancer cells TOV-112D CRL-11731 labeled with superparamagnetic scFv against HER2 harboring Gd atoms and imaged in Hitachi 3400 SEM with EDXSI. Secondary electron emission shows the cell surface ultrastructure (left). X-ray radiation at the specific for Gd atoms energy determines presence of scFv antibodies (middle). Gated elemental spectrum for Eu extracted from a pixel acquired with the beam parked over the scFv antibody chelated with Gd. Horizontal field width 65 microns.

Figure 2. Immunoblot of the ovarian cancer cells TOV-112D CRL-11731 and CRL-11732 OV-90 (lanes 1-2) and and breast cancer cells CRL-2340 HCC2157 lysates were electrotransfered onto PVDF membrane and labeled with the anti HER2/neu scFv without (left) and with (right) chelating Gd or Eu atoms. Intentionally the space below and above the bands are not cut to show absence of any non-specific binding, but only specific bands are present. Chelation did not change the specificity of scFv antibodies.
site single bands were clear indications of high specificity of the engineered antibodies (Figure 2). All the combinations resulted in the same labeling patterns. Importantly, the blots demonstrated that no other proteins in the entire cell lysate were labeled with our Gd chelated GE antibodies. The antibodies retained specificity towards targeted receptors, even after Gd coordination. Moreover, the background was entirely label free.

The ultimate test for attaining the project objective was the effect, which superparamagnetic antibodies anchored to the receptors on cell surfaces might have on local relaxivity.

Table 1 shows data from a representative experiment. Refined measurements were conducted on wide-bore Bruker (Table 1). Importantly, we observed significant increase in water relaxivity \( r \). That resulted in change in relaxivity proportional to the number of Gd chelated scFv antibodies attached. Relaxivity of water protons was about 200 mM \(^{-1}\) s\(^{-1}\) at 9.4T. The high relaxivity have to result in MRI contrast changes at antibody concentrations as little as 0.1 \( \mu \)M, which is sufficient for imaging of receptors in vivo. It was demonstrated that the antibodies with Gd are capable of labeling cells \textit{in vitro}. In our studies in cell culture, we have observed a significant contrast-to-noise ratio (CNR) enhancement due to superparamagnetic antibodies. Therefore, these scFv-based receptor targeting contrast agents created a clinically relevant change in relaxivity detectable in NMR (Table 1).

To summarize, in this initial study, striking differences were noticed in the signal strength generated between unlabeled ECM, fibroblasts, ovarian and breast cancer cells after labeling with the scFv antibody chelating Gd – antibody guided contrast.

**DISCUSSION**

This work provides the proof of concept for using superparamagnetic antibodies in detecting differences in levels of gene expression products in cells \textit{in vitro} and \textit{in vivo}. In practice, labeling of cell receptors with scFv antibodies resulted

<table>
<thead>
<tr>
<th>Table 1. Differences in T1 relaxation times, between unlabeled physiological fluids and tissues versus GE paramagnetic antibodies labeled cells.</th>
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<tbody>
<tr>
<td>Water</td>
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<tr>
<td>Serum</td>
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<td>Detroit fibroblasts culture</td>
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<tr>
<td>Ovarian cancer TOV-112D CRL-11731</td>
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<tr>
<td>Ovarian cancer TOV-112D CRL-11731 + anti HER2/neu scFv\textsubscript{Gd}</td>
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<tr>
<td>Breast cancer CRL-2340 HCC2157</td>
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Measurements of T1 relaxation times change induced by GE paramagnetic antibodies in [s] by inversion recovery with 400MHz at 9.4T on 28mm wide-bore Bruker.
in a dramatic shortening of T1. It was proportional to the number of Gd or Eu atoms harbored by scFv antibodies and anchored to the cell surface receptors. The significant difference between the number of the receptors on surfaces of cancer and normal cells translated into the significant difference in the signal intensity between these cells. This work opens new avenues for in vivo studies involving antibody guided contrast.

Success of this work can be primarily attributed to the high specificity, affinity, and small size of the engineered scFv. Their high specificity resulted not only in heavy labeling of the HER2 neu receptors, but also in reduced non-specific labeling of other cells. Therefore, the signal to noise ratio was remarkably high. The high affinity of these antibodies was shifting the dynamic on/off balance; thus enhancing conditions for T1 acquisition. Finally, the small size of these antibodies helped in their penetration into the depth of the cell spheroid cultures, as well as, in their packing onto the receptors. That increase in packing or labeling density was also seen on the images from Phosphorimager, LSCM, and EDXSI. The labeling density was much higher with scFv, than it was with Fab or IgG. In this study, it translated into the significant concentration of Gd or Eu atoms on surfaces of the cells. Higher number of antibodies, each harboring Gd or Eu atoms, resulted in significant changes of the relaxivity reflected in shortening of T1 and strengthening of the generated signal. This will be perceived as the bright spots on the screen of MRI scanner.

Specific signal to background noise ratio is the main factor to discriminate, the structure labeled with the element tagged recombinant antibody guided contrast agent from the unlabeled structures surrounding it. Therefore, the primary objective of this effort was to bioengineer antibodies in such a way that they would generate label-free background i.e., no non-specific labeling. As described earlier and applied here, it has been accomplished by selecting clones using short receptor domain sequence libraries, purification prior to and after derivatization, evaluation of antibody affinity on native electrophoresis and blue blots, and validation of the data with EDXSI. This complex approach resulted in very specific localization of the superparamagnetic antibodies on targeted HER2/neu receptors.

Improved packing of Gd atoms into chelating domains may enhance local relaxivity of water. Here, it has been accomplished by engineering metal binding domains into the scaffold of GE antibodies. Contrary to all of the other methods of antibody derivatization based upon random incorporation of chelating agents, which are changing properties of these antibodies, in this work the highly specific domains are specific integral parts of superparamagnetic antibodies, but completely separate from antigen binding domains. Therefore, they retain their bio-kinetic properties and antibody binding properties after incorporation of Gd or Eu into their scaffolds. Further, affinity purification, which follows derivatization, secures elimination of all molecules, which might have altered properties.

To summarize, we demonstrated a proof of concept for using antibody guided contrast agents for evaluating gene expression products. They warrant pursuing studies involving superparamagnetic antibodies in vitro and in vivo. The conclusions outlined above should serve as guides for their streamlining into in vivo molecular imaging endeavors.
Above all, the main challenge, before engineering superparamagnetic antibodies for in vivo MRI, is to secure thermodynamic stability of Gd or Eu within chelating pockets. Harboring these metals within antibodies has to be extremely stable, so that release and/or transmetallation do not induce toxic effects.

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LITERATURE CITED


