2011 Project Abstract
For the Period Ending June 30, 2015

PROJECT TITLE: Evaluation of Switchgrass as Biofuel Crop
PROJECT MANAGER: Jim Eckberg
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2011, First Special Session, Chp. 2, Art.3, Sec. 2, Subd. 06c
M.L. 2014, Chapter 226, Section 2, Subdivision 19

APPROPRIATION AMOUNT: $120,000

Overall Project Outcomes and Results

There is concern that native switchgrass bred for bioenergy may become invasive in Minnesota prairies. This project showed that selecting switchgrass for larger size (biomass) can increase it competitive ability and exacerbate it impacts on other native prairie plants. Switchgrass populations with large seed were more vigorous and produced more biomass leading to larger impacts on prairie diversity. Breeding for small seed size and/or less seed set could mitigate negative effects on prairies. There was a direct tradeoff between biomass production and diversity in a restored prairie, greater biomass was associated with less prairie diversity. Biofuels from switchgrass should use small seeded switchgrass populations to balance production versus diversity goals of prairies. Finally, we determined that poplar buffers can reduce switchgrass biomass 69% and could serve as a management tool in limiting the spread of switchgrass biofuel cultivars.

We conducted 10 experiments in total. In a restored prairie (Ag and Energy Center in Staples, MN) we established 176 1 m^2 plots of cultivar and wild switchgrass populations (13 total populations) and monitored them for two or three years. We tested the impacts of switchgrass cultivars in a native prairie at Cedar Creek Ecosystem Science Reserve from 2012-2014 (241 0.64 m^2 plots) and 2013-2014 (244 1 m^2 plots). Supporting the field studies was a growth chamber test of germination of 12 wild and cultivar populations as well as a greenhouse study testing switchgrass cultivars effects on two native grasses. We also tested poplar buffers and mowing in managing switchgrass from 2012-2014 at the Ag and Energy Center.

Information from this project is being used to inform breeding strategies for reduced invasion risk. We are working with a switchgrass breeder and switchgrass germplasm from our project was re-incorporated into a national breeding program to support the development of cultivars with potentially less invasion risk. Results from this project will support the development of sustainable bioenergy systems in Minnesota that balance biodiversity and production.

Project Results Use and Dissemination

We have presented results from this project for diverse audiences of ecologists, agronomists and conservations including two presentations at the national Ecological Society of America conference (2012, 2013), three presentations for undergraduate interns at Cedar Creek Ecosystem Science Reserve (2012, 2013, 2014), poster presentations for switchgrass breeders and agronomists at the national conference "Switchgrass II" (2013) and Pioneer seed company symposium (2015), and a webinar for the Minnesota DNR – Conservation Science Chat Series (2015).

To date we have published one peer-reviewed paper in *Crop Science* "Switchgrass population and cold-moist stratification mediate germination" and a second paper is in later stages of revision "Competitive interactions of cultivar and wild switchgrass with native grasses" and will be submitted to *Invasive Plant Science and Management*. Two additional peer-reviewed papers will be produced from this project.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2011 Work Plan Final Report

Date of Status Update: 11/23/2015

Final Report

Date of Work Plan Approval: 6/23/2011

Project Completion Date: 6/30/2015 **Is this an amendment request?**_Yes__

Project Title: Evaluation of Switchgrass as Biofuel Crop

Project Manager: Jim Eckberg

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Location:

Counties Impacted: Statewide

Ecological Section Impacted: Lake Agassiz Aspen Parklands (223N), Minnesota and Northeast Iowa Morainal (222M), North Central Glaciated Plains (251B), Northern Minnesota and Ontario Peatlands (212M), Northern Minnesota Drift and lake Plains (212N), Northern Superior Uplands (212L), Paleozoic Plateau (222L), Red River Valley (251A), Southern Superior Uplands (212J), Western Superior Uplands (212K)

Total ENRTF Project Budget:	**ENRTF Appropriation $:**	120,000
	Amount Spent $:	**120,000**
	Balance $:	0

Legal Citation: M.L. 2011, First Special Session, Chp. 2, Art.3, Sec. 2, Subd. 06c
M.L. 2014, Chapter 226, Section 2, Subdivision 19

Appropriation Language:
$60,000 the first year and $60,000 the second year are from the trust fund to the Minnesota State Colleges and Universities System for Central Lakes College in cooperation with the University of Minnesota to determine the invasion risk of selectively bred native grasses for biofuel production and develop strategies to minimize the invasion potential and impacts on biodiversity. This appropriation is available until June 30, 2014, by which time the project must be completed and final products delivered.

Carryforward: The availability of the appropriations for the following projects are extended to June 30, 2015 (4) Laws 2011, First Special Session chapter 2, article 3, section 2, subdivision 6, paragraph (c), Evaluation of Switchgrass as Biofuel Crop;

I. PROJECT TITLE: Evaluation of Switchgrass as Biofuel Crop

II. PROJECT SUMMARY:

Native switchgrass has been selected and bred to establish dense, productive biofuel stands. This major advance in biofuel sustainability also poses a significant risk to native biodiversity; selectively bred switchgrass shares many characteristics that typify our most invasive species. Little is known about the invasion risk posed by selective breeding and hybridization of native grasses. Invasion risk assessment is urgently needed before high-yielding switchgrass cultivars are planted extensively for biofuel production in Minnesota. This information will support next generation biofuel by identifying specific switchgrass cultivars and management strategies to minimize invasion risk. We will integrate three focus areas:

- **Invasion Risk—** Little is known about the potential for improved switchgrass varieties to invade prairie and impact local biodiversity. We will evaluate invasion risk by comparing competitiveness of improved switchgrass cultivars versus a study control, local genotypes of switchgrass.
- **Risk Management—** We will develop recommendations for managing buffers to limit the spread of potentially invasive grass biofuel crops. We will evaluate mowing and buffers for managing switchgrass escapees; recommendations will balance effective control with management cost.
- **Biofuel Sustainability—** Invasion risk and impacts on native biodiversity is often overlooked as a critical consideration for biofuel crop sustainability. We will integrate information on invasion risk and biofuel production to determine the trade-offs associated with more productive but potentially more invasive biofuel crops.

III. PROJECT STATUS UPDATES:

Project Status as of December 31, 2011:

ACTIVITY 1:
***Seed Collection from Remnant Switchgrass Populations*:** Shelby Flint and Jim Eckberg obtained a permit (2011-37R) from the DNR Scientific and Natural Areas Program to collect switchgrass seed from prairie Scientific and Natural Areas. They successfully located and collected seed from remnant switchgrass populations in 14 Scientific and Natural Areas across east-central, southeast, southwest and Northwest Minnesota. We are currently threshing the seed and we will test seed germinability.

ACTIVITY 1- Experiment 1 &
ACTIVITY 2- Buffer Treatments
***Experimental Field Preparation*:** The Ag Center staff prepared the experimental field for “ACTIVITY 1- Experiment 1” and “ACTIVITY 2- Buffer Treatments”. Soybeans were grown and harvested from this field in 2011. The staff planted an oat cover crop (September 29, 2011) to minimize potential fertilization effects on future switchgrass plots from the 2011 soybean crop.

Project Status as of July 2012:

ACTIVITY 1:
Experiment 1 was established at the Ag Center. A total of 105 experimental plots were planted on June 1, 2012. Overall, there is high establishment of perennial grasses and forbs. Experiment 2 was established at Cedar Creek in June 2012. Two sub-experiments were established: 2A or “seed addition” and 2B or “seedling addition”. These two experiments will help us understand the factors that influence initial seedling establishment (2A, seed addition) and those factors that affect switchgrass after seedling establishment (2B, seedling addition).

ACTIVITY 2- Buffer Treatments
We planted the poplar buffer experiment on June 22, 2012. Due to low establishment of the poplar we have replanted many of the poplar cuttings.

Project Status as of December 2012:

ACTIVITY 1:
***Additional Seed Collection from Remnant Switchgrass Populations*:** Shelby Flint and Jim Eckberg obtained a permit (2012-44R) from the DNR Scientific and Natural Areas Program to collect switchgrass seed from prairie Scientific and Natural Areas. We successfully located and collected seed from remnant switchgrass populations in seven Scientific and Natural Areas across east-central, southeast, and southwest Minnesota (Lost Valley, St. Croix Savanna, Prairie Coteau, Iron Horse, Weaver Dunes, Kasota Prairie and Black Dog SNA). These additional collections will add to current seed (collections at the same SNA collected from in 2011) or provide new remnant populations for study.
Experiment 1 (Ag Center):
Following the establishment of the experimental plots at the Ag Center on June 1, 2012, we evaluated the initial establishment density of switchgrass (July 24) and the end-of-season plant community composition (September 6-7). We are currently analyzing this data to evaluate the establishment rate and early dominance of switchgrass cultivars as compared to remnant, native switchgrass. On September 7 we quantified leaf area index of plots with switchgrass cultivars to quantify whether and how much switchgrass cultivars rapidly establish a canopy and potentially shade out other native plants.
Experiment 2 (Cedar Creek):
For the seed addition sub-experiment 2A, we counted the end-of-season seedling density (September 23 – 29). In the seedling addition sub-experiment (2B), we measured the initial seedling size (June 12 – 14), frequency of leaf herbivory (leaf chewing by insects) in the mid-season (July 25 – 26), as well as end-of-season (August 28 – 30) seedling survival, size, and the frequency of herbivory.

ACTIVITY 2:
Buffer experiment:
Following poor initial establishment of poplars, we replaced poplars on July 13 and August 31. By October we achieved 95% establishment of poplar. Given the establishment of poplars, we will be able to proceed with our original experimental plan and timeline: Plant switchgrass in 2013 and evaluate the effects of shading from poplar on switchgrass establishment.
Mowing experiment:
The mowing treatments were established on September 17, 2012. A stand of ‘Forestburg’ switchgrass (planted in spring 2010) located within 5 miles of the Ag Center was selected as the site for this study. We established two experimental treatments: mowed versus un-mowed.

Presentation of project at national ecological conference:
Our research group was invited to present the results of this study at the 97th Annual Ecological Society of America Conference in Portland, OR. We presented our project at a symposium titled “Growing Risk: Assessing the Invasive Potential of Bioenergy”, organized by Aviva Glaser of the National Wildlife Federation. Our presentation included the initial data and results from the project and we provided a discussion of the broader concern that native species, such as switchgrass, could become invasive under intense selection for bioenergy production. Funding to participate in this meeting (ie. travel outside MN) was provided by the University of Minnesota (Department of Agronomy).

Amendment Request (07/31/2013)

Request for Project Extension (From 6/30/2014 to 12/23/2014)
Throughout the implementation of this project, we have been able to efficiently manage the budget and have had opportunities cut costs of performing research activities. For example, after the LCCMR study

was funded Jim Eckberg acquired an NSF IGERT fellowship through the University of Minnesota which fully supported him from September 2011-December 2012. Since he was responsible for coordinating and leading on much of the research, this resulted in large savings to the project. As a result, we were able to expand the project with additional studies that build on, and provide further insight to, the original grant objectives. While some of the research activities have been advanced rapidly, continued poor growth of the poplars (Activity 2) will postpone our introduction of switchgrass to spring 2014.

We are requesting an extension to timeline of the grant and presentation of final deliverables so that we can collect an additional season of data on the experiments established in 2012 and 2013.

Request for Approval of Experiments Established in 2013:
In 2012, we gathered data suggesting that several environmental factors (rainfall, competition from other plants, and insect herbivory) may significantly affect switchgrass establishment and possibly invasiveness. However, these were observational findings. Without experimental manipulation of these environmental variables we could not quantify the impacts of these factors on switchgrass growth and spread. Therefore, in the spring of 2013 we established two new experiments at Cedar Creek Ecosystem Science Reserve to experimentally test the observations we made in 2012. Further, we observed strong competitive effects of switchgrass cultivars in our experiment at the Ag and Energy Center. To confirm these results, we established an additional field trial with a similar experimental design as in 2012.

We have been able to establish these new experiments completely within the original grant budget; no new funds are being requested. Again, this has been possible due to our efficient use of grant funds and outside support (i.e. NSF fellowship) secured for J. Eckberg.

Amendment Approved: 05/09/2014

Project Status as of July 2013:

ACTIVITY 1:
Experiment 1 (Ag Center):
We established the second seed addition experiment at the Ag Center on June 11-12, 2013 (sub-experiment 1B) adjacent to our first seed addition experiment established in 2013 (sub-experiment 1A).

Experiment 2 (Cedar Creek):
We established our manipulative experiments at Cedar Creek Ecosystem Science Reserve in spring 2013. An experimental seed addition was established on May 15-17, 2013 to test the effects of water limitation and insect herbivory on seedling establishment (sub-experiment 2C). An experimental transplant addition was established on May 23-24, 2013 to test the effects of plant competition and insect herbivory on switchgrass growth (sub-experiment 2D).

ACTIVITY 2:
Buffer experiment:
The poplar are all established but still small given the initial establishment problems. Therefore, we postponed the introduction of switchgrass until spring 2014.

Mowing experiment:
The experiment was established in 2012 with a single mowing event. A second mowing event will occur in August 2013.

Project Status as of December 2013:

ACTIVITY 1:
Experiment 1 (Ag Center):
Sub-Experiment 1A:
We collected data on leaf area and structure of the plots, end-of-season plant community composition, and switchgrass flowering in each of the plots. These data will provide insight on long-term changes in plant community composition and structure as affected by switchgrass cultivars versus remnant switchgrass.

Sub-Experiment 1B
In 2013, we evaluated the initial establishment density of switchgrass, leaf area index, switchgrass plant size, and end-of-season plant community composition. At the end of the growing season we randomly selected 528 switchgrass plants across all plots so that we can determine their survival rate over the 2013 to 2014 winter. Data analysis is underway to evaluate the establishment rate and early dominance of switchgrass cultivars as compared to remnant, native switchgrass

Preparation of research manuscript:
Our group is currently developing a manuscript from the data collected in 2012 and 2013 from sub-experiments 1A and 1B. We plan to submit this manuscript to a peer-reviewed ecological journal prior to the next status update (July 2014).

Experiment 2 (Cedar Creek):
Sub-experiment 2A
We counted end-of-season switchgrass seedling density. We measured mid and late summer leaf area index of the plots to evaluate the effect of the disturbance treatment in 2012 on plant cover throughout 2013. We are analyzing these results to evaluate how readily switchgrass cultivars establish and invade remnant prairie.

Sub-experiment 2B
We measured switchgrass survival and leaf herbivory in the early, mid and late-summer. We also quantified switchgrass size. The percent cover surrounding each switchgrass plant was evaluated in the mid-summer. To evaluate the effects of switchgrass cultivars on plant community structure we measured leaf area index in the early and late summer. Taken as a whole, these data will provide insight on the effect of plant competition and insect herbivory on switchgrass survival and growth.

Sub-experiment 2C
In this experiment we are evaluating the effects of water limitation and insect herbivory on the initial establishment of switchgrass from seed. We have added switchgrass seed into plots and we experimentally manipulated water (water addition) and insect herbivory (insecticide). We quantified leaf area index of these plots in the early and late summer. Insect leaf herbivory was observed in mid and late summer. We measured the density and size of switchgrass seedlings in the late summer.

Sub-experiment 2D
The objective of this experiment is to determine the effect of insect herbivory and competition from prairie plants on switchgrass growth and flowering and evaluate how such ecological effects potentially differ among cultivar and remnant switchgrass. We transplanted switchgrass to field plots with manipulation of competition (disturbance) and insect herbivory (insecticide). We quantified the effect of the disturbance treatment on plant cover by measuring leaf area index in the early and late summer. Insect leaf herbivory was observed in the early and late summer. Finally, to determine switchgrass growth we measured initial plant size and plant size at the end of the growing season.

ACTIVITY 2:

Buffer experiment:
Throughout the 2013 growing season we controlled agricultural weeds in the plots. The plots are now ready for the introduction of switchgrass seed in spring 2014.

Mowing experiment:
Plots were mowed for a second time on September 10 2013. Assessment of mowing treatments will occur during the summer of 2014.

Project Status as of July 2014:

ACTIVITY 1:
Experiment 1 (Ag Center):
Sub-Experiment 1A:
No data was collected on this experiment in the spring of 2014. During the summer of 2012, we observed stark differences in the emergence of switchgrass in this field experiment. This motivated us to perform a germination test in a growth chamber in January 2014 to assess the germinability and dormancy of switchgrass.

Sub-Experiment 1B
On June 30, 2014 we evaluated over-wintering survival and switchgrass size on switchgrass plants which had been marked the previous fall. This experiment tests the effect of switchgrass on the biomass and community composition of switchgrass. However, it is less clear how switchgrass may compete with and affect specific species of native plants. We performed a greenhouse competition experiment from February-April 2014 to test this question.

Preparation of research manuscript:
Our group has held off on submitting a manuscript from the data collected in 2012 and 2013 so that we can combine those data with results from 2014. We plan to submit a manuscript to a peer-reviewed ecological journal based on our combined data from 2012-2014.

Experiment 2 (Cedar Creek):
Sub-experiment 2A
No data was collected on this experiment in the spring of 2014.

Sub-experiment 2B
We measured switchgrass overwintering survival on June 16-17, 2014.

Sub-experiment 2C
In this experiment we are evaluating the effects of water limitation and insect herbivory on the initial establishment of switchgrass from seed. In July 2014 we have continued to impose the water addition and insect herbivory (insecticide) treatments on these plots. We measured the density of switchgrass seedlings on June 20-23, 2014.

Sub-experiment 2D
The objective of this experiment is to determine the effect of insect herbivory and competition from prairie plants on switchgrass growth and flowering and evaluate how such ecological effects potentially differ among cultivar and remnant switchgrass. In June-July 2014 we have continued to impose the insect herbivory (insecticide) treatments on these plots. We determined switchgrass overwintering survival by evaluating plants on June 16, 2014. We quantified the effect of the disturbance treatment on plant cover by measuring leaf area index on July 10-15, 2014.

ACTIVITY 2:

Buffer experiment:

We seeded the switchgrass cultivar Trailblazer into all poplar plots at a rate of 10 PLS grams/ m2 on May 28, 2014.

Mowing experiment:
No data was collected on this experiment in the spring of 2014.

Project Status as of December 2014:
ACTIVITY 1:
Experiment 1 (Ag Center):
Sub-Experiment 1A:
This experiment tested the impact of switchgrass cultivars on the diversity of recently restored prairie. We measured final percent cover on August 6th to 7th, 2014. Species composition and biomass were determined by clipping 0.1m^2 of the plot on August 6th to 12th, 2014. We have collected and processed all field data. The field component of this project is complete.

Sub-Experiment 1B:
In this experiment we re-tested the potential for switchgrass cultivars to impact the diversity of restored prairies. We measured final percent cover on August 12th, 2014. Species composition and biomass were determined by clipping 0.1m^2 of the plot on August 12th to 13th, 2014. We have collected and processed all field data. The field component of this project is complete.

Preparation of research manuscript:
We have prepared a research manuscript based on our growth chamber germination trials in 2013 and 2014. This manuscript is in the final stages of revision and will be submitted to the journal *Crop Science*.

Experiment 2 (Cedar Creek):
Sub-experiment 2A
This study began in the spring of 2012 to test the establishment of switchgrass populations and the invasion risk of switchgrass cultivars. We completed our final seedling count on September 16th to 25th, 2014 as well as our final measurement of plant cover (leaf area index) on September 16th to 18th. All field sample collection is completed for this project.

Sub-experiment 2B
In this experiment, started in spring of 2012, we tested the growth and performance of switchgrass. We completed our final measurement of switchgrass size and herbivory on August 20th to August 26th. We also harvested the switchgrass to measure total plant biomass and harvested the entire prairie plot to evaluate the impact of switchgrass on the prairie plant community. All field sample collection is completed for this project.

Sub-experiment 2C
The original objective of this research project was to test the role of switchgrass population and ecosystem factors (water limitation and herbivory) in the establishment of switchgrass. We completed our final measurements of switchgrass seedling density, size and herbivory on September 11th to 17th, 2014. We also measured the biomass of switchgrass and prairie. Field sample collection and sample processing are now complete for this project.

Sub-experiment 2D
This experiment tested the effect of plant competition and insect herbivory on switchgrass performance in the tallgrass prairie. We completed final measurements of switchgrass size, herbivory and flowering on August 29th to September 30th, 2014. All switchgrass and prairie plant species were clipped so that we could measure biomass. This marks the completion of all field sample collection and sample processing for this project.

ACTIVITY 2:

Buffer experiment:
We collected 15 switchgrass plants per plot to assess the effect of poplar on switchgrass biomass. All field collection of samples is completed for this project.

Mowing experiment:
We performed a final assessment of mowing treatments by evaluating average height of switchgrass in the mowed versus un-mowed treatments. The field evaluation of this project is now completed.

September 2015
Overall Project Outcomes and Results

There is concern that native switchgrass bred for bioenergy may become invasive in Minnesota prairies. This project showed that selecting switchgrass for larger size (biomass) can increase it competitive ability and exacerbate it impacts on other native prairie plants. Switchgrass populations with large seed were more vigorous and produced more biomass leading to larger impacts on prairie diversity. Breeding for small seed size and/or less seed set could mitigate negative effects on prairies. There was a direct tradeoff between biomass production and diversity in a restored prairie, greater biomass was associated with less prairie diversity. Biofuels from switchgrass should use small seeded switchgrass populations to balance production versus diversity goals of prairies. Finally, we determined that poplar buffers can reduce switchgrass biomass 69% and could serve as a management tool in limiting the spread of switchgrass biofuel cultivars.

We conducted 10 experiments in total. In a restored prairie (Ag and Energy Center in Staples, MN) we established 176 1 m^2 plots of cultivar and wild switchgrass populations (13 total populations) and monitored them for two or three years. We tested the impacts of switchgrass cultivars in a native prairie at Cedar Creek Ecosystem Science Reserve from 2012-2014 (241 0.64 m^2 plots) and 2013-2014 (244 1 m^2 plots). Supporting the field studies was a growth chamber test of germination of 12 wild and cultivar populations as well as a greenhouse study testing switchgrass cultivars effects on two native grasses. We also tested poplar buffers and mowing in managing switchgrass from 2012-2014 at the Ag and Energy Center.

Information from this project is being used to inform breeding strategies for reduced invasion risk. We are working with a switchgrass breeder and switchgrass germplasm from our project was re-incorporated into a national breeding program to support the development of cultivars with potentially less invasion risk. Results from this project will support the development of sustainable bioenergy systems in Minnesota that balance biodiversity and production.

Project Results Use and Dissemination

We have presented results from this project for diverse audiences of ecologists, agronomists and conservations including two presentations at the national Ecological Society of America conference (2012, 2013), three presentations for undergraduate interns at Cedar Creek Ecosystem Science Reserve (2012, 2013, 2014), poster presentations for switchgrass breeders and agronomists at the national conference “Switchgrass II” (2013) and Pioneer seed company symposium (2015), and a webinar for the Minnesota DNR – Conservation Science Chat Series (2015).

To date we have published one peer-reviewed paper in *Crop Science* “Switchgrass population and cold-moist stratification mediate germination” and a second paper is in later stages of revision “Competitive interactions of cultivar and wild switchgrass with native grasses” and will be submitted to *Invasive Plant Science and Management*. Two additional peer-reviewed papers will be produced from this project.

Amendment Request (11/23/2015):
During the completion of this project funds were shifted from publication to personnel. There were no costs associated with publishing the first peer-reviewed study from this project. Instead there were more expenses associated with personnel working on this project. This does not involve a shift of funds between activities but rather from publication to personnel (junior scientist) within each activity. This shift in funds occurred at the very end of the project when it became clear that funds would not be needed for publication but were needed to retain staff working on completing this project.

Approved by the LCCMR 12-8-2015

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Invasive Risk of Selectively Bred Switchgrass

Description:

Little is known about the invasion risk and impacts of switchgrass cultivars on native prairie plant diversity. We will conduct two separate studies to test the effect of switchgrass cultivars on prairie plant diversity in newly seeded prairies (Experiment 1) and established prairies (Experiment 2). Switchgrass cultivars will be compared to six locally native switchgrass populations (study control). This activity will occur in two locations at both the Ag and Energy Center of Central Lakes College in Staples, MN (Experiment 1) and Cedar Creek Ecosystem Science Reserve in East Bethel, MN (Experiment 2).

Seed:

In fall of 2011, we will collect switchgrass seed from 6 local native switchgrass populations within 100 miles of the Ag and Energy Center and Cedar Creek. We will minimize the risk of genetic contamination by non-local switchgrass in several ways. First, we will collect from large switchgrass populations that are surrounded by areas with well documented agricultural and planting history. Second, we will confirm that there are no known CRP plantings within 1 km of our collection. Our standards exceed the rigor of current Minnesota Crop Improvement Agency standards for verifying origin-identified (yellow tag) native plant material for use in prairie restorations (MCIA 2010). Further, any potential contamination will likely be small and/or diluted by large local switchgrass populations.

We will focus on three switchgrass cultivars. An evaluation of switchgrass cultivar establishment, underway in 2010 and 2011, will be used to refine the final list of cultivars to focus on a suite that differs in productivity and potential invasiveness. This could lead to recommendations for selecting cultivars that produce high biomass yields while minimizing invasion risks.

Using seed from the same three cultivars and six locally native populations of switchgrass we will conduct experiments on invasion risk and impacts in newly seeded (experiment 1) and established prairie (experiment 2)

In experiment 1, we will use MnDOT seed mixture 350 (includes grasses and forbs) to establish a mixture of prairie species. This mixture is established widely across the state for prairie restorations. Switchgrass seed will be assigned to one of six seed densities (see below). Seed will be cold stratified in the winter prior to seeding in spring 2012.

Experiment 1 Design:

This study will involve a full factorial of the treatments: Cultivar (3 types) versus locally native switchgrass (6 types) (9 total, referred from here on as “switchgrass types”) and 6 seed densities (25, 50, 100, 150, 250, and 350 seeds/ m^2). Four m^2 plots of each switchgrass type and seed density will be established in the spring of 2012. Switchgrass seed will be incorporated with the MnDOT seed mixture 350 and be distributed evenly over each plot. Seed will be raked into the top 1 cm of soil to improve seed-to-soil contact and simulate conventional seed drilling techniques used to restore prairies. As a part of conventional management for newly seeded prairies, we will perform two to three herbicide treatments prior to seeding and we will control annual weed cover using a combination of mowing, hand-weeding and/or clipping during both years of the study (2012-2013).

We will replicate cultivar (3 types) vs. non-cultivar (6 types) plots at a 2:1 ratio. This will allow for more power to detect differences between specific cultivars whereas non-cultivars are considered random samples of a larger pool; therefore, we increase the number of native switchgrass populations versus replication within each population. The experimental design will be repeated in two sites (72 plots/ site; 144 plots total) at the Ag and Energy Center.

Measurements:

In the first year of establishment (2012), we will harvest above-ground plant material in a 0.5 m^2 subplot within each 4 m^2 plot to determine the biomass of each species. Biomass will be collected at the end of the growing season and before senescence to allow for identification and sorting of species. In year 2 (2013, post-establishment) we will repeat the same measurements. Our response variables for switchgrass are biomass (percent switchgrass biomass). Our response variables for the entire plant community-plot are species richness and percent composition. We will use these data to calculate diversity indices for each plot.

Statistical Analysis:

This study will be analyzed with a linear mixed effect model. Cultivar versus locally native switchgrass will be treated as fixed effects whereas each locally native switchgrass population (6) will be treated as random effects. Locally native switchgrass populations represent random samples of a larger statistical population (central Minnesota) thereby satisfying the requirements of a random effect. In contrast, each switchgrass cultivar will be treated as a fixed effect because information on selected growth characteristics of each cultivar will allow for specific, testable predictions of invasiveness among cultivars. This analysis will allow us to make specific inferences regarding the invasiveness of each cultivar. Our design includes more experimental units for each cultivar to improve statistical power to detect differences among cultivars. Site will be considered a random effect.

Seed density will be analyzed as a continuous fixed effect. We have chosen a wide range of seed inputs for several reasons. First, because a mixed model compares the slope and intercepts of a line fit to the data, added data along the line is a better use of experimental units as compared to more data at each point along the line. Second, this approach will shed more light on the relationship between arriving seed number and switchgrass dominance. A single seed level experiment could miss critical insight; the impacts of switchgrass cultivars are likely to vary with seed input and density dependence. Using Akaike’s information criterion, we will test the fit of competing models that describe the shape of the curves.

Experiment 2 Design:

As in experiment 1, experiment 2 will involve a full factorial of the treatments (fixed effects): Cultivar versus locally native switchgrass (9 total types) and 6 seed densities (25, 50, 100, 150, 250, and 350 seeds/ m^2). Four m^2 plots of each switchgrass type and seed density will be established in two established prairies during the spring of 2012. The experimental design will be replicated within two sites (144 plots/ site; 288 plots total) at Cedar Creek. If the study is not conducted at Cedar Creek, we will use a suitable alternative location.

Seeding and plot establishment methods will mimic a seed dispersal event into an established prairie. Therefore, in contrast to experiment 1, we will evenly distribute the seeds over each plot but we will not rake the seeds into the soil. Ideally we will establish the plots in fall 2011. However, seed collection will also occur in September and October of 2011; therefore, an early snowfall may prevent seeding in fall 2011. If seeding is postponed until spring 2012, seeds will be cold stratified during the winter.

This study will be analyzed using the same model structure as in experiment 1, a linear mixed effect model.

Measurements:

In the first year of establishment (2012), we will harvest above-ground plant material in randomly selected 0.5 m^2 subplots (within each 4 m^2 plots) and determine the biomass of switchgrass and other prairie species. Biomass will be collected at the end of the growing season and before senescence to allow for identification and sorting of species. In year 2 (2013, post-establishment) we will repeat the same measurements in a different randomly selected subplot. Our response variables for the entire plant community-plot are species richness and percent composition. We will use these data to calculate diversity indices for each plot.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 95,000
Amount Spent: $ 95,000
Balance: $ 0

Activity Completion Date:

Outcome	**Completion Date**	**Budget**
1. Collect seed from remnant switchgrass populations	November 1, 2011	$7,000
2. Establish and maintain experimental plots and treatments	September 15, 2013	$43,000
3. Collect, process and analyze data (2012-2013 growing seasons)	February 1, 2014	$38,000
4. Submit manuscript(s) to peer-reviewed journals	June 1, 2014	$7,000

Activity Status as of December 31, 2011:

Seed Collection from Remnant Switchgrass Populations**:** Shelby Flint and Jim Eckberg obtained a permit (2011-37R) from the DNR Scientific and Natural Areas Program to collect switchgrass seed from prairie Scientific and Natural Areas. Between September 9 – 26, 2011, S. Flint and J. Eckberg successfully located and collected seed from switchgrass populations in 14 Scientific and Natural Areas: Felton Prairie (Shrike Unit), Sandpiper Prairie, Black Dog Nature Preserve, Lost Valley Prairie, St. Croix Savanna, Uncas Dunes, Helen Allison Savanna, Rushford Sand Barrens, Joseph A. Tauer Prairie, Lundblad Prairie, Prairie Coteau, Western Prairie, Verlyn Marth Memorial Prairie, and Ottertail Prairie.

Within the Scientific and Natural Areas (SNA) we used a GPS to record the UTM for each seed collection location. We collected switchgrass within an approximate 30 meter radius of each UTM center point. Between four to 14 collections were obtained for each SNA. We collected seeds as far away from roads and/or the SNA boundaries as practical to minimize the potential for contamination (pollination) by non-local switchgrass genotypes. We did not collect seed from SNAs that were close to or bordered by a Wildlife Management Area given their extensive history of prairie reconstruction and the potential for cross contamination. We will use the UTM coordinates to develop a map to verify that our collection locations are within the SNA boundaries. This map will be submitted to the DNR and LCCMR.

We have threshed almost all the switchgrass seed. Next we will separate the chaff from the seed and test the seeds viability (germination and dormancy rates).

***Preparation of Experimental Field*:** The experimental plots for "ACTIVITY 1- Experiment 1" will be established at the Ag and Energy Center in Staples, MN. In August we identified a 0.885 acre field suitable for our experimental plots: homogenous topographic and soil features, non-irrigated, low weed pressure, and convenient access. Soybeans were grown on this plot in 2011. Ag Center staff harvested the soybeans on September 26, 2011. The soybeans were direct cut by a 17.5' combine header, run through the combine, and the chaff was distributed evenly across the field. The chaff was disked into the top six inches of the soil during seedbed preparation for the oats. We planted an oat cover crop (2 bushels/ ac) on September 29, 2011 to remove residual nitrogen and minimize potential fertilization effects on the experimental switchgrass plots next spring (2012).

Activity Status as of July 2012:

For activity 1, we established experiment 1 (June 1, 2012) and experiment 2 (June 6-15, 2012). For experiment 2 we conducted an additional sub-experiment which involved the planting of switchgrass seedlings. Below we provide the details on the experimental set-up for experiments 1 and 2.

Activity 1, Experiment 1: On June 1, 2012, we established all plots for experiment 1 at the Agriculture and Energy Center in Staples, MN. We established a fully factorial experiment using nine remnant populations of switchgrass and four switchgrass cultivars. The cultivars included Trailblazer and Summer as well as advanced biofuel cultivars (EG-2101 and WS12L-IL). WS12L-IL is one of the most advanced switchgrass biofuel cultivars; it has not been commercially released.

Seed Source		**Seed Density Treatments**		**Replication**		**Number of 1.0 m^2 Plots**
9 Native Minnesota	**X**	Five	**X**	One	**=**	45
4 Cultivars	**X**	Five	**X**	Three	**=**	60
				Total	**=**	105

Five switchgrass seed density treatments (0, 50, 150, 450, and 1350 seeds/ 1 m^2) were added and raked into each plot. Also, a constant background mixture of four native grasses (Canada Wild Rye, Big Bluestem, Side Oats Grama, and Indian Grass) and five native forbs (Black-eyed Susans, Purple Prairie Clover, Sky Blue Aster, Anise Hyssop and Stiff Goldenrod) was seeded in each plot at a rate of 4.69 PLS grams per 1 m^2 plot.

On July 24, 2012 we quantified emergence of switchgrass and other seedlings in the plots. We are currently analyzing these data.

Activity 1, Experiment 2: We established two separate sub-experiments which we refer to as sub-experiment 2A (seed addition) and sub-experiment 2B (seedling addition).

Sub-experiment 2A (seed addition): We established all plots for the seed addition sub-experiment at the Cedar Creek Ecosystem Science Reserve on June 6, 2012. We established a fully factorial experiment using nine remnant populations of switchgrass and three switchgrass cultivars. The cultivars included Trailblazer and Summer as well as an advanced biofuel cultivar, EG-2101.

Seed Source		**Seed Density Treatments**		**Disturbance Treatment**	**Replication**		**Number of 0.64 m^2 Plots**
9 Native Minnesota	X	Five	X	Disturbance vs. Control	One	=	90
3 Cultivars	X	Five	X	Disturbance vs. Control	Four	=	120
					Total	=	210

Five switchgrass seed density treatments (0, 50, 150, 450, and 1350 seeds/0.64 m^2) were added and raked into each plot. Half of the plots were disturbed by light tilling and drilling holes. This experiment will show us how well switchgrass invades in established prairie in relation to switchgrass cultivar, seed density and disturbance.

Sub-experiment 2B (seedling addition): To quantify the impacts of established switchgrass cultivars on prairie plant communities we planted switchgrass seedlings in prairie. To start the seedlings seed from each of the switchgrass cultivars and native Minnesota varieties were planted in the University of Minnesota greenhouse in early April. The resulting seedlings were then trans-planted at the Cedar Creek Ecosystem Science Reserve on June 15, 2012. We established our experiment using 13 remnant populations of switchgrass and four switchgrass cultivars. The cultivars included Trailblazer and Summer as well as an advanced biofuel cultivars, EG-2101 and WS12L-IL. We only established a single plot for seven native Minnesota varieties because there was a limited number of emerging switchgrass seedlings in the greenhouse.

Seedling Source		**Density Treatments**		**Replication**		**Number of 0.64 m^2 Plots**
Pseudo-Plantings	X	Four	X	Five	=	20
6 Native Minnesota	X	Four	X	One	=	24
7 Native Minnesota	X	One	X	One	=	7
4 Cultivars	X	Four	X	Five	=	80
				Total	**=**	**131**

Four switchgrass seedling density treatments (0, 1, 7 and 20 seedlings/0.64 m^2) were planted in each plot. This experiment will help us quantify the impacts of switchgrass on prairie in relation to switchgrass plant density and variety.

Activity Status as of December 2012:

Experiment 1 (Ag Center):
Following the planting of the experimental plots at the Ag Center on June 1, 2012, we evaluated the initial establishment density of switchgrass (July 24). We placed a small frame (10 x 10 cm) to sub-sample each plot, and identified and counted all grass and forb species. We are currently analyzing these data to evaluate whether and how much switchgrass cultivars establish more rapidly than remnant switchgrass.

We quantified end-of-season plant community composition (September 6-7). We placed a one m^2 frame propped up to a height of 20 cm above each plot. Within the frame, we identified plant species at every point along a string grid consisting of 16 points (4 x 4 points). We calculated the percent cover of each species in each plot as the number of times we encountered that species in the grid divided by the total number of points for each plot (16). Currently, we are analyzing these data to evaluate the establishment rate and early dominance of switchgrass cultivars as compared to remnant, native switchgrass.

On September 7, we quantified leaf area index of the plots using a light meter (AccuPAR LP-80). Leaf area index data can be used to determine how rapidly switchgrass cultivars develop a leaf canopy. We are currently analyzing these results to determine if switchgrass cultivars establish a canopy more quickly than remnant switchgrass. If so, switchgrass cultivars may shade-out other native plants.

Experiment 2 (Cedar Creek):
For the seed addition sub-experiment 2A, we counted end-of-season switchgrass seedling density (September 16 – 29) in the center of each plot (40 x 40 cm). We also measured leaf area index of the plot to evaluate the effect of the disturbance treatment on plot plant cover (September 11). We are analyzing these results to evaluate how readily switchgrass cultivars establish and invade remnant prairie.

In the seedling addition sub-experiment 2B, we measured the initial seedling size in the greenhouse prior to planting (June 12 – 14); measurements included the number of stems, length of the longest leaf, and number of leaves. By early July we observed some striking patterns in leaf herbivory (leaf chewing by insects) in the field experiment. We then quantified the frequency of leaf herbivory in the mid-season (July 25 – 26) as the proportion of switchgrass with greater than 9% leaf removal (versus less than 9% leaf removal). We repeated the measurements of seedling survival, size, and frequency of herbivory at the end of the season (August 28 – 30). We are evaluating this data to determine how the interactions of switchgrass with insect herbivores differ between cultivar and remnant switchgrass. Differences in herbivory may have important implications for the invasiveness of switchgrass cultivars.

Preparation of research manuscript:
Our group is currently developing a manuscript from the data collected in 2012. We plan to submit this manuscript to a peer-reviewed ecological journal prior to the next status update (July 2013).

Activity Status as of July 2013:

For activity 1, we established sub-experiments 1B (June 11-12, 2013), 2C (May 15-17, 2013), and 2D (May 23-24, 2013). Below we provide the details on the experimental set-up.

Activity 1, Sub-Experiment 1B: On June 11-12 (2013), we established all plots for sub-experiment 1B at the Agriculture and Energy Center in Staples, MN. We established a fully factorial experiment using seven remnant populations of switchgrass and two switchgrass cultivars. The cultivars included ‘Summer’ as well as advanced biofuel cultivar ‘EG-2101’.

Seed Source		Seed Density Treatments		Replication		Number of 1.0 m^2 Plots
7 Native Minnesota	X	Three	X	Two	=	42
2 Cultivars	X	Three	X	Four	=	24
				Total	=	66

Three switchgrass seed density treatments (100, 450, and 1350 seeds/ 1 m^2) were added and raked into each plot. Also, a constant background mixture of four native grasses (Canada Wild Rye, Big Bluestem, Side Oats Grama, and Indian Grass) and five native forbs (Black-eyed Susans, Purple Prairie Clover, Sky Blue Aster, Anise Hyssop and Stiff Goldenrod) were seeded in each plot at a rate of 4.69 PLS grams per 1 m^2 plot. This experiment will provide an additional cohort of switchgrass to further test our original hypothesis that switchgrass cultivars establish more readily and dominate in restored tallgrass prairie plant communities.

Activity 1, Experiment 2: We established two new sub-experiments: Manipulation of water and insect herbivory effects on establishment of seedlings from seed (sub-experiment 2C) and manipulation of plant competition and insect herbivory effects on growth of switchgrass transplanted from the greenhouse (sub-experiment 2D).

Sub-experiment 2C (Seed addition with experimental manipulation of water and herbivory): We established all plots for the seed addition sub-experiment 2C at the Cedar Creek Ecosystem Science Reserve on May 15-17, 2013. We established a fully factorial experiment using four remnant populations of switchgrass and one switchgrass cultivar, EG-2101. Switchgrass was planted at a single, high seed density (2500 seeds/ m^2).

Seed Source		Herbivory Treatments		Water Addition Treatment	Replication		Number of 1 m^2 Plots
4 Native Minnesota	X	Insecticide vs. Control	X	Water Addition vs. Control	Three	=	48
1 Cultivar	X	Insecticide vs. Control	X	Water Addition vs. Control	Seven	=	28
					Total	=	76

We learned in the 2012 that switchgrass is dependent on soil disturbance to establish and, so, all plots were disturbed (light tilling and uniformly dug holes with soil auger) prior to introducing the switchgrass seed. In half of the experimental plots, we regularly (every 2-3 weeks) apply a broad spectrum insecticide (bifenthrin) to reduce insect herbivory and evaluate the effects of insect herbivory on switchgrass survival and growth. Application of insecticide was approved by the Cedar Creek Review Board. In half of the experimental plots we add water (~50 increase in annual rainfall) to evaluate the effects of water limitation on switchgrass seedling survival and growth. This experiment will show us how important insect herbivory and water limitation are to switchgrass establishment in tallgrass prairie.

Sub-experiment 2D (Switchgrass transplant addition with experimental manipulation of plant competition and herbivory): We tested the effects of plant competition (via a disturbance) and insect herbivory (insecticide treatment) on the survival and growth of switchgrass cultivars and remnant populations in tallgrass prairie. To start, the seed from each of the switchgrass cultivars and native Minnesota populations were grown in the University of Minnesota greenhouse. The resulting seedlings were then transplanted to the Cedar Creek Ecosystem Science Reserve on May 24, 2013. We established our experiment using eight remnant populations of switchgrass and two switchgrass cultivars. The cultivars included EG-2101 and WS12L-IL. Four switchgrass plants were planted per one m^2 plot.

Seed Source		Herbivory Treatments		Disturbance Treatment	Replication		Number of 1 m^2 Plots
8 Native Minnesota	X	Insecticide vs. Control	X	Disturbed vs. No Disturbance	Three	=	96
2 Cultivars	X	Insecticide vs. Control	X	Disturbed vs. No Disturbance	Nine	=	72
					Total	=	168

Half of the plants are treated with insecticide following the same protocol as in sub-experiment 2C. Half of the plots are disturbed; disturbance involved tilling the plots prior to planting the switchgrass. This experiment will allow us to experimentally quantify the effects of plant competition and herbivory on switchgrass survival and growth in prairie and determine how critical these factors are to several switchgrass genotypes.

Activity Status as of December 2013:

Experiment 1 (Ag Center):
Sub-Experiment 1A:
On June 11-12 and August 21-22, we quantified leaf area index of the plots using a light meter (AccuPAR LP-80). We quantified end-of-season plant community composition (August 21-22) in 2013 using the same protocol as in 2012. On August 22, we determined the amount of switchgrass flowering in each of the plots. These data will provide insight on long-term changes in plant community composition and structure as affected by switchgrass cultivars versus remnant switchgrass.

Sub-Experiment 1B
Following the planting of the second seed addition experimental plots at the Ag Center on June 11, 2013, we evaluated the initial establishment density of switchgrass (August 5 and 7). To do this, we placed small frames (10 x 10 cm) within each half of the plot and identified and counted all grass and

forb species. On August 21, we quantified leaf area index of the plots using a light meter (AccuPAR LP-80). Leaf area index data can be used to determine how rapidly switchgrass cultivars develop a leaf canopy. Finally, we quantified end-of-season plant community composition (August 22) using the same approach as sub-experiment 1A. Data analysis is underway to evaluate the establishment rate and early dominance of switchgrass cultivars as compared to remnant, native switchgrass

At the end of the growing season (October 11-23) we randomly selected 528 switchgrass plants across all plots and measured their size (number of stems and leaves and length of the longest leaf). We marked these plants so that we can determine their survival rate over the 2013 to 2014 winter. Measurements of plant size and survival will help explain how potential differences in plant size contribute to differences in plant community composition among plots with switchgrass cultivars versus remnant switchgrass.

Preparation of research manuscript:
Our group is currently developing a manuscript from the data collected in 2012 and 2013 from sub-experiments 1A and 1B. We plan to submit this manuscript to a peer-reviewed ecological journal prior to the next status update (July 2014).

Experiment 2 (Cedar Creek):
Sub-experiment 2A
We counted end-of-season switchgrass seedling density (September 13-17) in the center of each plot (40 x 40 cm). On June 19 and September 16-17 we measured leaf area index of the plots to evaluate the effect of the disturbance treatment in 2012 on plant cover throughout 2013. We are analyzing these results to evaluate how readily switchgrass cultivars establish and invade remnant prairie.

Sub-experiment 2B
We measured switchgrass survival and leaf herbivory in the early (June 17-24), mid (July 23-25) and late-summer (September 3-6). On September 3-6 we also quantified switchgrass size (number of stems and leaves and the length of the longest leaf). In the mid-summer (July 23-25) we visually estimated the plant cover and competition surrounding each switchgrass plant as low (0-25% cover), medium (26-50% cover) or high (≥ 51% cover). To evaluate the effects of switchgrass cultivars on plant community structure we measured leaf area index in the early (June 28) and late summer (September 4-6). Taken as a whole, these data will provide insight on the effect of plant competition and insect herbivory on switchgrass survival and growth. Differences in such effects among switchgrass cultivars and remnant switchgrass may have important implications for the potential invasiveness of switchgrass cultivars.

Sub-experiment 2C
In this experiment we are evaluating the effects of water limitation and insect herbivory on the initial establishment of switchgrass from seed. We have added switchgrass seed into plots and we experimentally manipulated water (water addition) and insect herbivory (insecticide). We quantified leaf area index of these plots in the early (June 18) and late (August 29) summer. Insect leaf herbivory was observed in mid (July 10-11) and late (August 26-29) summer. We measured the density and size (number of stems and leaves and length of the longest leaf) of switchgrass seedlings in the late summer (August 26-29). As a whole, this experiment is providing insight on the ecological factors that limit initial switchgrass establishment and how such ecological interactions differ for switchgrass cultivars and remnant populations.

Sub-experiment 2D
The objective of this experiment is to determine the effect of insect herbivory and competition from prairie plants on switchgrass growth and flowering and evaluate how such ecological effects potentially differ among cultivar and remnant switchgrass. In this experiment we transplanted switchgrass to field plots with manipulation of competition (disturbance) and insect herbivory (insecticide). We quantified the effect of the disturbance treatment on plant cover by measuring leaf area index in the early (June 20-24) and late summer (August 16-19). Insect leaf herbivory was observed in the early (June 26) and late summer (August 12-19). Finally, to determine switchgrass growth we measured initial plant size

(May 22-23) and plant size at the end of the growing season (August 12-19). We are using these data to evaluate the ecological factors that limit the growth and reproduction of switchgrass after the initial establishment of switchgrass.

Summary of data collection to date: To facilitate the tracking of data collection across the six sub-experiments established for Activity 1 we provide a table of the sub-experiments, type of data collected, and years in which the particular data collection were performed (below). A dash indicates that we did not collect the data. In most cases data was not collected because it was either not relevant to the experimental objectives or we chose to collect those data from an experiment that was more appropriately designed to provide those data.

Sub- Experiments (year of experiment establishment)	Leaf area index	Switchgrass Seedling Density	Switchgrass Survival	Switchgrass plant size and growth	Insect herbivory on switchgrass	Switchgrass Flowering	Switchgrass percent cover
1A: Ag Center invasion test by switchgrass seed in restored prairie (2012)	2012, 2013	2012	-	-	-	2012, 2013	2012, 2013
1B: Ag Center invasion test by switchgrass seed in restored prairie with cold-moist stratification of seed (2013)	2013	2013	2013	2013	-	2013	2013
2A: Cedar Creek test of invasion by switchgrass seed in native prairie (2012)	2012, 2013	2012, 2013	-	-	-	2012, 2013	-
2B: Cedar Creek test of invasion by switchgrass transplants in native prairie (2012)	2012, 2013	-	2012, 2013	2012, 2013	2012, 2013	2012, 2013	-
2C: Cedar Creek test of invasion by switchgrass seed in native prairie with manipulation of rainfall and insect herbivory (2013)	2013	2013	-	2013	2013	2013	-
2D: Cedar Creek test of invasion by switchgrass transplants in native prairie with	2013	-	2013	2013	2013	2013	-

manipulation of plant competition and insect herbivory (2013)

Project Status as of July 2014:

ACTIVITY 1:
Experiment 1 (Ag Center):
Sub-Experiment 1A:
No data was collected on this experiment in the spring of 2014.

During January 2014 we performed a germination trial in the growth chamber to test the dormancy and response to cold-moist stratification. We selected three cultivars and five remnant populations of switchgrass. We exposed each genotype to a cold-moist stratification (cold-wet conditions) and control treatments (n=5 petri dishes per treatment; 50 seeds per petri dish). We monitored the petri dishes for germination over two weeks. Remaining seeds were dissected and exposed to tetrazolium to assess viability. These results provide important insight on the role of switchgrass population and cold-moist stratification to switchgrass emergence.

Sub-Experiment 1B
On June 30, 2014 we evaluated over-wintering survival and switchgrass size on switchgrass plants which had been marked the previous fall. In total 528 plants were marked, we measured survival on all plants and we measured switchgrass size (longest leaf length, number of stems, and number of leaves) on a subset of 198 plants.

From February to April 2014 we performed a greenhouse competition experiment to test the competitive effects of EG-2101 and Trailblazer on the performance of side-oats grama and Canada wild rye. Four densities of switchgrass (0, 3, 9, or 18 switchgrass/ pot) were planted around the focal plant (side-oats grama or Canada wild rye). We compared the competitive effects of EG-2101 and Trailblazer versus that of five remnant switchgrass populations. There were two replications per cultivar.

Preparation of research manuscript:
Our group has held off on submitting a manuscript from the data collected in 2012 and 2013 so that we can combine those data with results from 2014. We plan to submit a manuscript to a peer-reviewed ecological journal based on our combined data from 2012-2014.

Experiment 2 (Cedar Creek):
Sub-experiment 2A
No data was collected on this experiment in the spring of 2014.

Sub-experiment 2B
We measured switchgrass overwintering survival on all plants during June 16-17, 2014.

Sub-experiment 2C
In this experiment we are evaluating the effects of water limitation and insect herbivory on the initial establishment of switchgrass from seed. During June-July 2014 we continued to impose the water addition and insect herbivory (insecticide) treatments on these plots. We measured the density of switchgrass seedlings surviving the winter on June 20-23, 2014.

Sub-experiment 2D

The objective of this experiment is to determine the effect of insect herbivory and competition from prairie plants on switchgrass growth and flowering and evaluate how such ecological effects potentially differ among cultivar and remnant switchgrass. In June-July 2014 we continued to impose the insect herbivory (insecticide) treatments on these plots. We determined switchgrass overwintering survival by evaluating plants on June 16, 2014. We quantified the effect of the disturbance treatment on plant cover by measuring leaf area index on July 10-15, 2014.

Activity Status as of December 2014:
ACTIVITY 1:
Experiment 1 (Ag Center):
Sub-Experiment 1A:
This experiment tested the impact of switchgrass cultivars on the diversity of recently restored prairie. We measured final percent cover on August 6th to 7th, 2014. Species composition and biomass were determined by clipping 0.1m^2 of the plot on August 6th to 12th, 2014. We have collected and processed all field data.

Sub-Experiment 1B:
In this experiment we re-tested the potential for switchgrass cultivars to impact the diversity of restored prairies. We measured final percent cover on August 12th, 2014. Species composition and biomass were determined by clipping 0.1m^2 of the plot on August 12th to 13th, 2014. We have collected and processed all field data.

Preparation of research manuscript:
We have prepared a research manuscript based on our growth chamber germination trials in 2013 and 2014. This manuscript is in the final stages of revision and will be submitted to the journal *Crop Science*.

Experiment 2 (Cedar Creek):
Sub-experiment 2A
This study began in the spring of 2012 to test the establishment of switchgrass populations and the invasion risk of switchgrass cultivars. We completed our final seedling count on September 16th to 25th, 2014 as well as our final measurement of plant cover (leaf area index) on September 16th to 18th. All field sample collection is complete.

Sub-experiment 2B
In this experiment, started in spring of 2012, we tested the growth and performance of switchgrass. We completed our final measurement of switchgrass size and herbivory on August 20th to August 26th. We also harvested the switchgrass to measure total plant biomass and harvested the entire prairie plot to evaluate the impact of switchgrass on the prairie plant community. All field sample collection is complete.

Sub-experiment 2C
The original objective of this research project was to test the role of switchgrass population and ecosystem factors (water limitation and herbivory) in the establishment of switchgrass. We completed our final measurements of switchgrass seedling density, size and herbivory on September 11th to 17th, 2014. We also measured the biomass of switchgrass and prairie. Field sample collection and sample processing are now complete.

Sub-experiment 2D
This experiment tested the effect of plant competition and insect herbivory on switchgrass performance in the tallgrass prairie. We completed final measurements of switchgrass size, herbivory and flowering on August 29th to September 30th, 2014. All switchgrass and prairie plant species were clipped so that we could measure biomass. This marks the completion of all field sample collection and sample processing.

Final Report Summary:

ACTIVITY 1:

Throughout this project we have strived to provide the greatest value for the funds. We expanded these experiments (from 5 original experiments to 10 experiments) to develop a more comprehensive understanding of potential invasion risk, we sought out a switchgrass breeder to integrate our results with breeding programs to provide meaningful recommendations for reduced invasion risk, and we have actively presented these results to a relevant audience of plant breeders, conservationists (DNR) and ecologists. We will continue to publish results from this project to advance the science of breeding biomass crops with less risk of invasion.

Experiment 1 (Ag Center):
One peer-reviewed paper has been accepted for publication in the journal *Crop Science*. The study is title "Switchgrass population and cold-moist stratification mediate germination". This experiment supported our field studies by providing information on dormancy of switchgrass cultivars and wild populations. Overall, cultivars showed lower dormancy as compared to wild populations yet there was large variation in germination among wild populations. This variation correlated positively with seed size. Further, cold-moist conditions stimulated germination of virtually all switchgrass populations. This study suggests that lower dormancy and larger seed size contributed to greater establishment of switchgrass cultivars and some wild populations.

A second paper is in late stages of revision "Competitive interactions of cultivar and wild switchgrass with native grasses". This study focused on testing the effects of switchgrass cultivars on two common native prairie plants (sideoats grama and Canada wild rye). We found that switchgrass cultivars reduced sideoats grama biomass much more so than Canada wild rye. This information is useful in supporting our wider efforts to understand the community level effects of switchgrass cultivars on native prairies in the field. This paper also represents a joint collaboration with a switchgrass breeder using ecological data to inform breeding strategies. Switchgrass populations with large seed were more aggressive, therefore breeding for small seed size may limit invasion risk.

For the field studies, we completed data processing and are in the early stages of analysis. The results show that switchgrass cultivars have greater emergence and attain greater percent cover than wild populations in the first year of a prairie restoration. However, by the second and third years cultivars were not significantly more productive than wild populations. This shift in results came after extremely cold winters. Cultivars were expected to be hardy for USDA hardiness zones 4 but it seems likely that harsh winter conditions contributed to their lower performance. This was a potential setback as the original project goal was to evaluate cultivars that were winter hardy so that we could consider invasive potential from selecting for increased biomass production. Despite this, other interesting patterns have emerged from the study. In particular, among the wild populations (all originating from Minnesota), large seeded populations showed much greater biomass and greater impacts on prairie biomass.

Selecting switchgrass for larger size (biomass) can increase it competitive ability and exacerbate it impacts on other native prairie plants. Switchgrass populations with large seed were more vigorous and produced more biomass leading to larger impacts on prairie diversity. Breeding for small seed size and/or less seed set could mitigate negative effects on prairies. There was a direct tradeoff between biomass production and diversity in the restored prairie, greater biomass was associated with less prairie diversity. Biofuels from switchgrass should use small seeded switchgrass populations to balance production versus diversity goals of prairies.

We have partnered and are collaborating with a USDA-ARS switchgrass breeder to integrate information on switchgrass invasion into breeding programs to support the development of less invasive switchgrass cultivars. Our study suggests that large seeded switchgrass cultivars can establish in restored prairie and have greater impacts on prairie biomass (potentially diversity) than small seeded populations. This suggests that breeding for reduced seed set and seed size can limit the potential for switchgrass cultivars to spread from biomass production fields and impact prairies. Breeding for delayed flowering and less seed set is a current breeding strategy for switchgrass being employed in breeding programs. Our results reinforce the value of such a strategy for reducing spread and impacts of switchgrass cultivars into prairie.

Experiment 2 (Cedar Creek):

We completed data processing and are in the early stages of analysis. There are several key findings from these studies. Switchgrass cultivars showed greater establishment densities yet similar to Experiment 1 these effects did not persist after the first year. Again, this may be due to low winter hardiness in switchgrass cultivars. Among the wild populations, there was also a trend toward larger seeded populations producing more biomass and potentially being more aggressive. These results reinforced the importance of developing switchgrass with less seed set, as establishment from seed tended to lead to greater switchgrass establishment. Another key finding is that cultivars, in general, were attacked by insect herbivores (e.g. grasshoppers) more often than wild populations. This may be due to less plant defense associated with breeding for greater biomass. These results suggest that cultivars are not universally more invasive; in fact, they may be less invasive than wild populations owing to their greater susceptibility to herbivory.

In this project we studied a lowland (low latitude) switchgrass population which has been selected for later flowering times to reduce seed set and increase biomass production. Lowland switchgrass varieties show significant potential for reduced flowering, greater biomass production from an extended growing season and less invasion due to lower seed set. A major barrier to developing such populations is their lack winter hardiness. Our project contributed significantly to helping overcome winter hardiness by exposing a population of these plants to the farthest north winter conditions (Cedar Creek, East Bethel, MN) to date. The winter survivors were then re-incorporated into a national breeding program to support the development of cultivars with delayed flowering, less seed set, and potentially less invasion risk. We will produce one to two publications from Experiment 2 that will further inform breeding programs for reduced invasion risk.

ACTIVITY 2: Invasion Risk Management

Description:

We will test two strategies for controlling switchgrass cultivars: mowing and buffer composition. These two studies will occur at the Ag and Energy Center of Central Lakes College in Staples, MN.

Mow Treatments:

We will test the efficacy of mowing to control switchgrass cultivars. Mow treatments will involve cutting established stands of Forestburg switchgrass to a height of 5-10 cm. Through a Next Gen grant, one acre plots of Forestburg switchgrass were established at the Center in 2009. Within each of these large plots we will establish small (4 m^2) mowing treatment plots. Within each switchgrass stand, we will assign the following three treatments: no mow (control), mowed in early summer, mowed in late summer, and mowed in both early and late summer. Mowing times are designed to target specific life history stages: early summer and late summer mowing removes basal and flowering stalks (plus basal leaves), respectively. Consecutive within-season mowing has been shown to reduce switchgrass vigor (Cuomo, Anderson et al. 1998) but the potential for mowing to reduce invasiveness remains less clear. This experiment will be replicated in three switchgrass stands (12 total experimental units). We will perform these treatments in 2012.

Mowing Measurements:

We will measure height and biomass of switchgrass in the spring of 2013 for 0.5 m^2 subplots within each plot to assess the effects of mowing treatments in 2012 on subsequent regrowth in 2013. A final measurement of switchgrass biomass will be collected after senescence in the fall of 2013. The data will be analyzed with ANOVA.

Buffer Treatments:

We will test the effect of aspen (*Populus tremuloides*) windrows in preventing the spread of switchgrass cultivars. Aspen was selected as the windrow species because it is fast-growing and common in north-central Minnesota. Our windrow consists of three rows of trees, 6 meter width, and 20 meter height. For this study, the windrow will be located to the east of the plots. Future research will test windrows located to the north, south, and west of plots.

Three switchgrass cultivars will be planted in four m^2 plots (250 seeds/ m^2) during the spring of 2012 at varying distances from the edge of the aspen windrow (8 meters, 2 meters, windrow edge, and 2 meters into the windrow). We will control weeds in these plot areas prior and after seeding to isolate the direct effect of the windrow on switchgrass growth. By varying planting distances in relation to the windrow we intend to mimic a dispersal event of seeds into the windrow, and test the capacity of windrows to suppress the establishment and spread of switchgrass. The same three cultivars used in activity #1 will be used in this study. Each set of distances will be replicated four times per switchgrass cultivar (3 cultivars X 4 distances X 4 replications = 48 experimental units). This simple design will allow us to evaluate the efficacy of windrow buffers in minimizing switchgrass cultivar spread via seed dispersal.

Buffer Measurements:

In the first year of establishment (2012), we will measure establishment as the biomass of switchgrass harvested in 0.5 m^2 subplots at the end of the growing season. In fall of 2013 we will harvest senesced switchgrass in a different randomly selected subplot. We will evaluate the impacts of the windrow by regular measurements of light penetration across the season and in relation to distance to the windrow. The data will be analyzed with ANOVA.

Summary Budget Information for Activity 2: **ENRTF Budget: $ 25,000**
Amount Spent: $ 25,000
Balance: $ 0

Activity Completion Date:

Outcome	**Completion Date**	**Budget**
1. Establish and maintain experimental plots and treatments	September 15, 2013	$17,500
2. Collect, process and analyze data (2012-2013 growing seasons)	February 1, 2014	$6,900
3. Submit manuscript to peer-reviewed journal	June 1, 2014	$600

Activity Status as of December 31, 2011:

***Preparation of Experimental Field*:** Plots for “ACTIVITY 2 – Buffer Treatments” will be established in the same experimental field as described for “ACTIVITY 1 – Experiment 1”. Refer to Activity 1 status on 12/31/2011 for details on field preparation.

Activity Status as of July 2012:

Buffer Treatments:
We established four experimental poplar plots on June 22, 2012. Each plot consisted of a 5 x 12 planting block with poplar at 1 m spacing. In 2013, switchgrass will be planted within the poplar and adjacent to the poplar to assess the effects of poplar on switchgrass establishment. Due to low establishment of the poplar, we have replanted over half of the poplar cuttings.

Mow Treatments:
We will perform our experimental mowing in August 2012.

Activity Status as of December 2012:

Buffer experiment:
Following poor initial establishment of poplars, we replaced poplars on July 13 and August 31. To establish the poplar we received guidance and plant material from Bernie McMahon of the University of Minnesota Duluth- Natural Resources Research Institute. The experiment now consists of two cultivars of poplar (*DN5* provided by B. McMahon and *99059016* provided by Mike Young of Verso Paper). These two cultivars are spread evenly across the experimental plots. Using these two cultivars we achieved 95% establishment of poplar by October. Now that we have established the poplars, we will be able to proceed with the experiment. This will include planting the switchgrass in 2013 and evaluating the effects of shading from poplar on switchgrass establishment.

Mowing experiment:
The mowing treatments were established on September 17, 2012. A stand of 'Forestburg' switchgrass (planted in spring 2010) located within 5 miles of the Ag Center was selected as the site for this study. We established two experimental treatments: mowed versus unmowed. In total there were eight experimental plots (four plots per treatment), each plot was 100 ft^2 (10 x 10 ft). In summer 2013 we will quantify the regrowth of the plots to evaluate the effects of mowing on switchgrass persistence.

Activity Status as of July 2013:

Buffer experiment:
Throughout the 2013 growing season we have controlled agricultural weeds in the plots in preparation for the introduction of switchgrass seed in spring 2014.

Mowing experiment:
Plots will be mowed for a second time in August 2013. Assessment of mowing treatments will occur during the summer of 2014.

Activity Status as of December 2013:

Buffer experiment:
Throughout the 2013 growing season we controlled agricultural weeds in the plots. The plots are now ready for the introduction of switchgrass seed in spring 2014.

Mowing experiment:
Plots were mowed for a second time on September 10 2013. Assessment of mowing treatments will occur during the summer of 2014.

Activity Status as of July 2014:

Buffer experiment:
We seeded the switchgrass cultivar Trailblazer into all poplar plots at a rate of 10 PLS grams/ m2 on May 28, 2014. The seeding rates and seed bed preparation (scuffle hoes and raking) were similar

amongst the poplar and open areas of the plots. To plant these plots we 1) removed weeds from all areas of the plot, 2) raked all areas of the plot to produce a uniform seed bed, 3) seeded the plot at a rate of 10 PLS grams/ m2 in the areas including the poplar and open spaces. This experiment will allow us to test whether poplar buffers can limit the invasion potential of switchgrass.

Mowing experiment:
No data was collected on this experiment in the spring of 2014.

Project Status as of December 2014:
Buffer experiment:
We collected 15 switchgrass plants per plot to assess the effect of poplar on switchgrass biomass. All field collection of samples is completed for this project.

Mowing experiment:
We performed a final assessment of mowing treatments by evaluating average height of switchgrass in the mowed versus un-mowed treatments.

Final Report Summary:

The buffer experiment revealed that poplar strips can reduce individual switchgrass biomass by 69%. This is striking given how immature the poplar still were (6-10 ft) and this suggests such windrows are an effective means to limiting switchgrass from spreading out of biomass production fields.

The mowing had little effect on the final height of switchgrass at the end of the 2014 season. Mowing may have been too infrequent to reduce switchgrass biomass. Regardless, because mowing is time intensive, impacts other species, and does not eradicate switchgrass, it is unlikely to be an effective management strategy.

V. DISSEMINATION:

Description:
Results and recommendations from this study will be disseminated to farmers and conservation, industry, university, and government organizations through the following outlets:

- Field demonstration days are conducted annually in August. Tours of the plots will be conducted to discuss our experimental research and implications for biofuel production and natural areas.
- Annual updates of our research will be provided through the Center's website (http://www.clcmn.edu/agcenter/index.html).
- Seminars and presentations will be given for the Minnesota DNR, Ecological Society of America, the University of Minnesota and other potential Universities and/or government agencies.
- Publishable results from this research will be submitted to peer-reviewed journals.

Status as of July 2012

As of July 1, 2012 no dissemination has occurred for this project.

Status as of December 2012

Presentations:
97th Annual Ecological Society of America Conference in Portland, OR
We were invited to present the project at a symposium titled “Growing Risk: Assessing the Invasive Potential of Bioenergy”. The symposium was organized by Aviva Glaser of the National Wildlife Federation. We presented the initial results emerging from this experiment and provided a discussion of the broader concern that native species, such as switchgrass, could become invasive under intense selection for bioenergy production.

Cedar Creek Summer Seminar Series
On July 3rd, we were invited to present the project to approximately 60 undergraduate interns working at Cedar Creek Ecosystem Science Reserve.

Status as of July 2013

Cedar Creek Research Symposium:
On June 20th, 2013, we presented the project to approximately 60 undergraduate interns, graduate students, and faculty working at Cedar Creek Ecosystem Science Reserve.

Status as of December 2013

98th Annual Ecological Society of America Conference in Minneapolis, MN
On August 8th, we presented our results as a part of the “Environmental Impact and Risk Assessment” contributed oral session. We presented the complete results from our 2012 season and we provided a description of the ecological risk surrounding the intense selection and breeding of bioenergy crops.

Switchgrass Conference II in Madison, WI
We presented a research poster of our results to an audience that spanned the diverse range of stakeholders involved in the growing, breeding, and development of switchgrass as a bioenergy crop.

Status as of July 2014:

Cedar Creek Research Symposium:
On June 23rd, 2014, we presented the project to approximately 60 undergraduate interns, graduate students, and faculty working at Cedar Creek Ecosystem Science Reserve.

Status as of December 2014:
No presentations have occurred since the last update. We are currently working with Jason Garms and Mark Lindquist of the DNR to host a webinar for DNR employees statewide. The objectives of the presentation, tentatively set for March 2014, will be to discuss the risk of using switchgrass cultivars versus wild populations in restored prairies and as a source of biofuel. We will also compare the risk of switchgrass biofuel crops based on our research to risk known from literature on other biofuel crops (e.g. Miscanthus).

Final Report Summary:

We have presented results from this project for diverse audiences of ecologists, agronomists and conservations including two presentations at the national Ecological Society of America conference (2012, 2013), three presentations for undergraduate interns at Cedar Creek Ecosystem Science Reserve (2012, 2013, 2014), poster presentations for switchgrass breeders and agronomists at the national conference “Switchgrass II” (2013) and Pioneer seed company symposium (2015), and a webinar for the Minnesota DNR – Conservation Science Chat Series (2015).

We have produced one peer-reviewed paper accepted in April 2015 for publication in *Crop Science* "Switchgrass population and cold-moist stratification mediate germination". A second paper is in later stages of revision "Competitive interactions of cultivar and wild switchgrass with native grasses" and will be submitted to Invasive Plant Science and Management. Two to three additional peer-reviewed papers will be produced from this project.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget:

Budget Category	$ Amount	Explanation
Personnel:	$ ~~96,859~~ $ 98,359	**Robert Schafer -** Supervisor **-** Agricultural and Energy Center within Central Lakes College. Responsible for managing the budget and expenses for this grant (MNSCU); contribute to experimental plot set-up and management; oversee outreach effort. Annual Salary $65,156 plus fringe $16,289 x 0.03 FTE = $2,443 x 3 years **(total = $7,330).** **Ron Nelson** - Farm Manager - Agriculture and Energy Center within Central Lakes College. Contribute to experimental site selection, preparation, and plot management. Annual Salary $37,547 plus fringe $9,387 x 0.06 FTE = $2,816 x 3 years **(total = $8,448).** **Undergraduate Interns -** University of Minnesota and Central Lakes College. Establish and maintain plots, collect and process data samples. 4000 total hours @ $11 / hr **(total = $44,000).** **Junior Scientist -** University of Minnesota. Refine experimental protocol; oversee plot establishment, data collection and processing; analyze and interpret data; write and submit manuscripts. Annual Salary $39,600 plus fringe $14,572 x 0.32 FTE x 2.08 yr **(total = ~~$34,551~~ $36,051).** **Matt Bickel** - Lead Technician - University of Minnesota. Provide GIS expertise to locate remnant switchgrass populations. Annual Salary $36,122 plus fringe $14,485 x 0.05 FTE **(total = $2,530).**
Equipment/Tools/Supplies:	$5,211	**Supplies:** Switchgrass and prairie seed mixture (480 experimental plots) **(total = $2,500)**. **Supplies to:** establish plots (ie tape meters, rakes, herbicide, etc.), maintain plots (ie scissors, gloves, surveyor flags, metal tags), assist in seed organization (ie. envelopes), and collect and process data (ie. notebooks, meter sticks) **(total = $1,661)**. **Equipment Use:** Fuel and maintenance expenses for spraying, tractor, and brush cutter equipment used to establish and maintain plots **(total = $1,050)**.
Printing:	$500	Printing for annual agriculture field days and other demonstrations

Travel Expenses in MN:	$14,430	Travel from Saint Paul to Staples and Cedar Creek research plots; lodging and food reimbursements associated with travel to Staples (Expenses adhere to UMN travel expense policy). (Activity 1: 18,000 total miles X $0.51/mile = $9,180, lodging and food reimbursements = $2,720. Activity 2: 3,000 miles X $0.51/mile = $1,530, lodging and food reimbursements = $1,000)
Other: Soil Analysis	$1,500	Soil Analysis @ Soil Testing Lab, Crops Research Building, University of Minnesota. (ie. Analysis of one sample for total nitrogen and organic carbon, pH, potassium, phosphate and total phosphorus = $61 x 24 samples = $1464)
~~Other: Publication Costs~~	~~$1,500~~	~~Peer-reviewed Journal Publication Cost (ie. Ecological Society of America charges $75/printed page X 6-8 pages per article = $600/ publication)~~
TOTAL ENRTF BUDGET:	**$120,000**	

Explanation of Use of Classified Staff: Robert Schafer and Ron Nelson are classified staff. However, they are not provided with an annual, regular salary from the state. Their salary is generated from on-farm and activities such commodity production and grants (soft money).

Explanation of Capital Expenditures Greater Than $3,500: N/A

Number of Full-time Equivalent (FTE) funded with this ENRTF appropriation: 2.98 FTE

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
	$0	$0	
State			
	$0	$0	
TOTAL OTHER FUNDS:	**$0**	**$0**	

VII. PROJECT STRATEGY:
A. Project Partners:

Partners Receiving Funds from the ENRTF appropriation:

Robert Schafer (Supervisor – Agriculture and Energy Center, Central Lakes College) **$7,330**
Shelby Flint (Graduate Student – University of Minnesota) **$12,017**

Robert Schafer will serve as co-project manager. He will manage the budget and expenses for this grant (MNSCU), contribute to experimental plot establishment and weed management, and he is responsible for outreach and dissemination of information to the agricultural community. Shelby Flint will refine and adapt the experimental protocols, oversee plot and treatment establishment, data collection and analysis as well as prepare research results for publication in peer-reviewed journals. Shelby will provide two years (@ 0.15 FTE) in-kind service and she will be partially supported with funds from the ENRTF appropriation for one year (0.32 FTE).

Partners Providing In-Kind Services:

Shelby Flint (Graduate Student – University of Minnesota) **0.15 FTE x 2 years**

Jim Eckberg (Graduate Student – University of Minnesota) **0.15 FTE x 3 years**

Jim Eckberg will serve as co-project manager and he will partner with Shelby Flint to lead this study. They will refine and adapt the experimental protocols, oversee plot and treatment establishment, data collection and analysis as well as prepare research results for publication in peer-reviewed journals. Shelby Flint and Jim Eckberg will provide in-kind services for two and three years, respectively.

University of Minnesota Partners Providing Advisory Services:

Neil Anderson (Associate Professor, Horticultural Science)
Gregg Johnson (Associate Professor, Agronomy and Plant Genetics)
Nicholas Jordan (Professor, Agronomy and Plant Genetics)
Ruth Shaw (Professor, Ecology, Evolution and Behavior)
Craig Sheaffer (Professor, Agronomy and Plant Genetics)
Donald Wyse (Professor, Agronomy and Plant Genetics)

Our team of agronomists and ecologists from the University of Minnesota will provide guidance and input on the refinement of research protocols, data analysis, and research manuscripts resulting from this grant. Our group has expertise in agronomy and biofuel production, plant breeding, and ecology and we are unified by the central goal of developing productive biofuel systems that do not threaten the state's native biodiversity.

B. Project Impact and Long-term Strategy:

High-yielding grasses provide new opportunities for sustainable biofuel production on marginal lands. However, there is increasing concern that these fast-establishing grasses may invade and impact natural areas. This research will provide one of the first invasion risk assessments of biofuel crops in Minnesota; such information is central for breeders, conservationists, and agronomists working to expand biofuel production without causing large-scale plant invasions in natural areas. Thus we have assembled a team a faculty members with expertise spanning agronomy, plant breeding and ecology.

At the core of this project is the Energy and Agricultural Center of Central Lake College in Staples, Minnesota. The Center is working to develop a community-based and sustainable biofuel industry for the Central Sand Plains. In 2009, the Center secured a Next Gen grant to evaluate the production of perennial grasses, including Miscanthus x giganteus and switchgrass. The current LCCMR is a direct extension of the Next Gen grant and will allow us to evaluate invasive potential of switchgrass at the Center. We are currently pursuing federal funds to expand and extend the LCCMR-funded research. In particular we intend to quantify dispersal distance and the dispersal kernel of switchgrass seeds. Contingent on federal funding we will construct spread-impact models for switchgrass as well as continue monitoring our plots to track long-term changes in prairie diversity and productivity.

C. Spending History:

Funding Source	**M.L. 2005 or FY 2006-07**	**M.L. 2007 or FY 2008**	**M.L. 2008 or FY 2009**	**M.L. 2009 or FY 2010**	**M.L. 2010 or FY 2011**
	$0	$0	$0	$0	$0

VIII. ACQUISITION/RESTORATION LIST: N/A

IX. MAP(S): N/A

X. RESEARCH ADDENDUM: See Research Addendum (Revised May 2011)

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted not later than December 15, 2011, July 15, 2012, December 15, 2012, July 15, 2013, December 15, 2013, July 31, 2014 and December 15, 2014. A final report and associated products will be submitted by June 1st, 2015.

Final Attachment A: Budget Detail for M.L. 2011 (FY 2012-13) Environment and Natural Resources Trust Fund Projects

Project Title: Evaluation of Switchgrass as Biofuel Crop
Legal Citation: M.L. 2011, First Special Session, Chp. 2,
Project Manager: Robert Schafer and Jim Eckberg
M.L. 2011 (FY 2012-13) ENRTF Appropriation: $120,000
Project Length and Completion Date: June 2015
Date of Update: December 8 2015

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised Activity 1 Budget 11/23/2015	Amount Spent	Balance	Revised Activity 2 Budget 11/23/2015	Amount Spent	Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	*Invasion Risk and Impacts of Selectively Bred Switchgrass*				*Invasion Risk Management*			
Central Lakes College and University of Minnesota Personnel	*77400*	*77400*	*0*	*20959*	*20959*	*0*	98359	0
Personnel: Robert Schafer - Supervisor - Agricultural and Energy Center within Central Lakes College, Responsible for managing the budget and expenses for this grant (MNSCU); contribute to experimental plot set-up and management; oversee outreach effort. Annual Salary $65,156 plus fringe $16,289 x 0.03 FTE = $2,443 x 3 years **(total = $7,330)**.		*4330*			*3000*			
Personnel: Ron Nelson - Farm Manager - Agriculture and Energy Center within Central Lakes College. Contribute to experimental site selection, preparation, and plot management. Annual Salary $37,547 plus fringe $9,387 x 0.06 FTE = $2,816 x 3 years **(total = $8,448)**.		*5955*			*2500*			
Personnel: Undergraduate Interns - University of Minnesota and Central Lakes College . Establish and maintaining plots; collect and process data samples. 4000 total hours @ $11 / hr **(total = $44,000)**.		*36700*			*9120*			
Personnel: Junior Scientist - University of Minnesota . Coordinate experimental plot establishment, plot maintenance, data collection and processing. Annual Salary $39,600 plus fringe $14,572 x 0.32 FTE = $17,335 x 2.08 years **(total = $36,051)**.		*30415*			*4100*			
Personnel: Matt Bickel - Lead Technician - University of Minnesota. Provide GIS expertise to locate remnant switchgrass populations. Annual Salary $36,122 plus fringe $14,485 x 0.05 FTE **(total = $2,530)**.		*0*			*2239*			
Supplies: Switchgrass and prairie seed mixture (480 experimental plots)	*2,000*	*2,000*	*0*	*500*	*500*	*0*	2,500	0
Supplies: Various Supplies to: establish plots (ie. tape meters, rakes, herbicide, etc.), maintain plots (ie. scissors, gloves, surveyor flags, metal tags), assist in seed organization (ie. envelopes, bags), and collect and process data.	*1,500*	*1,500*	*0*	*161*	*161*	*0*	1,661	0
Equipment Use: Fuel and maintenance expenses for spraying, tractor, and brush cutter equipment used to establish and maintain plots	*800*	*800*	*0*	*250*	*250*	*0*	1,050	0
Printing - Annual Agriculture field days and other demonstrations	*400*	*400*	*0*	*100*	*100*	*0*	500	0
Travel expenses in Minnesota - Travel from Saint Paul to Staples and Cedar Creek research plots; lodging and food reimbursements associated with travel to Staples (Expenses adhere to UMN travel expense policy). (**Activity 1**: 18,000 total miles X $0.51/mile = $9,180, lodging and food reimbursements = $2,720. **Activity 2**: 3,000 miles X $0.51/mile = $1,530, lodging and food reimbursements = $1,000)	*11,900*	*11,900*	*0*	*2,530*	*2,530*	*0*	14,430	0
Other: Soil Analysis @ Soil Testing Lab, Crops Research Building, University of Minnesota (ie. Analysis of one sample for total nitrogen and organic carbon, pH, potassium, phosphate and total phosphorus = $61 x 24 samples = $1464)	*1,000*	*1,000*	*0*	*500*	*500*	*0*	1,500	0
COLUMN TOTAL		$95,000	$0		$25,000	$0	120,000	$0

Published October 16, 2015

RESEARCH

Switchgrass Population and Cold–Moist Stratification Mediate Germination

James O. Eckberg,* Michael D. Casler, Gregg A. Johnson, Laura L. Seefeldt, Karen E. Blaedow, and Ruth G. Shaw

ABSTRACT

Switchgrass (*Panicum virgatum* L.) breeding and selection have enhanced the agronomic qualities of this species as a crop for forage and bioenergy applications. Previous work has characterized variation in phenotypic traits (e.g., survival, biomass yield, and cell wall carbohydrates) among wild and cultivated populations. Despite the importance of low dormancy to the establishment of a productive switchgrass stand, there is little information characterizing the dormancy of selectively bred cultivars vs. wild populations of switchgrass. The objectives of this study were to use growth chamber experiments to quantify germination vs. dormancy (confirmed by tetrazolium tests) of eight wild and four cultivar populations and evaluate the relationship between seed size and germination. While cultivars generally showed higher germination than wild populations, there was marked variation in germination among wild populations; for those with lower germination, the ungerminated fraction comprised mostly live (i.e., dormant) seeds. These data led us to perform a subsequent experiment testing the application of a seed treatment, cold–moist stratification, on a subset of eight populations representing the wide variation in germination observed in the first experiment. Cold–moist stratification substantially increased germination, but the magnitude of the effect varied among populations. Populations with higher dormancy showed a much larger increase in germination after cold–moist stratification. These data clearly show that seed dormancy in wild populations can be easily overcome by cold–moist stratification in the short term and breeding and selection in the long term.

J.O. Eckberg, G.A. Johnson, L.L. Seefeldt, and Karen E. Blaedow, Dep. of Agronomy and Plant Genetics, Univ. of Minnesota, Saint Paul, MN 55108; M.D. Casler, USDA–ARS, US Dairy Forage Research Center, 1925 Linden Dr., Madison, WI 53706; and R.G. Shaw, Dep. of Ecology, Evolution and Behavior, Univ. of Minnesota, Saint Paul, MN 55108. Received 24 Feb. 2015. Accepted 27 May 2015. *Corresponding author (jeckberg@umn.edu).

Abbreviations: AOSA, Association of Official Seed Analysts; DNR, Department of Natural Resources; SNA, Scientific and Natural Areas Program.

The development of perennial grasses for bioenergy and biomaterial production is a current challenge for agronomists and geneticists. For nearly 70 yr, the native warm-season switchgrass has been the focus of breeding and selection, first for forage and conservation applications and more recently for bioenergy production (Casler, 2012). Because of the large net-energy yields and potential for future improvement (Schmer et al., 2008), switchgrass is poised to support the Renewable Fuel Standards and Energy Independence and Security Act of 2007 (USEPA, 2010). Despite major gains in switchgrass agronomics and breeding (McLaughlin and Kszos, 2005; Casler, 2012), several limitations must be addressed to improve the efficiency of switchgrass-based biomass production systems. Switchgrass seed dormancy can be high, sometimes over 80% (Blake, 1935; Sanderson et al., 1996; Shen et al., 2001), and this can limit the establishment and long-term productivity of the crop (Mitchell and Vogel, 2012). While variation in other agronomic traits (e.g., biomass yield, survival, and cell wall carbohydrates) has been characterized (Casler, 2005), similar studies are lacking for germination and dormancy.

Breeding and selection programs have presumably reduced dormancy in switchgrass cultivars because dormant seeds are eliminated by successive cycles of selection (Casler, 2012). For

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example, four cycles of direct selection against dormancy increased germination of the cultivar 'Alamo' by 16 to 71%, depending on the seed lot and germination conditions (Burson et al., 2009). Germination is also greater for heavier seed (Aiken and Springer, 1995; Smart and Moser, 1999) and selection for large seed size has led to the release of cultivars with enhanced germination and seedling emergence (Boe and Ross, 1998). The relative effects of seed size per se vs. selection history on germination have not been directly quantified. Studies on germination in switchgrass have been limited to cultivars only (Zarnstorff et al., 1994; Aiken and Springer, 1995; Smart and Moser, 1999; Shen et al., 2001), all of which likely underwent some selection for less dormancy (Casler, 2012). More insight on the factors affecting germination could be realized by a broader comparison of cultivars vs. wild populations, which have little to no artificial selection for less dormancy. Such an evaluation could inform breeding strategies by providing insight on the extent to which selection and large seed size are associated with reduced dormancy in cultivars and wild populations.

The application and potential benefits of seed treatments on germination could be better understood in the broader context of population-level variation in switchgrass germination. Many seed treatments have been investigated as a means to alleviate dormancy in switchgrass including cold–moist stratification, scarification (mechanical and chemical), variable storage durations and conditions, and growth hormones (Zarnstorff et al., 1994; Haynes et al., 1997; Madakadze et al., 2000). Chemical treatments, while effective at increasing germination (Haynes et al., 1997; Madakadze et al., 2000), can be costly or impractical when applied to commercial-scale biomass production. Cold–moist stratification is both practical and effective at enhancing germination (Zarnstorff et al., 1994; Shen et al., 2001). However, little is known about how the effect of cold–moist stratification differs among wild and cultivated populations that vary in dormancy.

In this study, we compare the proportion of germinating vs. dormant seeds of four cultivars and eight wild switchgrass populations and evaluate how variation in seed size among switchgrass populations influences germination (Experiment 1). The four cultivars have a known history of selection for agronomic traits or have undergone seed multiplication; dormancy is one of the first wild traits to be reduced in breeding programs (Casler, 2012). All eight wild populations were collected from prairie in the Minnesota Scientific and Natural Areas (SNAs) Program in Minnesota and have little to no artificial selection. We then evaluate the interactive effect of population and cold–moist stratification on germination and dormancy (Experiment 2).

MATERIALS AND METHODS

Switchgrass Seeds: Wild Populations

Both experiments used switchgrass seed collected from wild populations and cultivars. From 11 to 24 Sept. 2011, switchgrass seed was collected from eight prairies, each considered a separate wild population, in central and southern Minnesota. One population (Black Dog collection area) represented seed collected in 2011 and 8 Sept. 2012. We minimized the potential for genetic contamination by cultivar seed or cross-pollination by collecting populations (i) from prairie in the SNAs program (Minnesota Department of Natural Resources and Minnesota Chapter- the Nature Conservancy) that represent remnant plant populations or reseeded populations collected from remnant prairie within 40 km, (ii) avoiding SNAs that were adjacent to Department of Natural Resources (DNR) Wildlife Management Areas with large-scale prairie reconstructions, and (iii) making every practical effort to collect seed close to the interior of the SNA. Within each SNA, switchgrass seed was collected from four to eight locations (30 m max. search radius). Based on GIS analysis, only one of 46 collection points was located outside the SNA, 68 m away in a plant community similar to the sampled SNA. All other collection areas were located 40 to 413 m away from the edge of each SNA. Finally, we reviewed Minnesota DNR management notes and 1991 aerial photos for evidence of field cultivation and reseeding, as such populations may have reduced dormancy (Casler, 2012). Two of the eight populations were reseeded using remnant prairie seed sources within 40 km, while the others are likely remnant populations.

Switchgrass Seeds: Cultivars

We studied three upland switchgrass cultivars: Summer (Kaste Seed Inc.), Trailblazer (Stock Seed Farms), and EG-2101 (CERES Blade), as well as a lowland cultivar selected for biomass production, WS12L-IL (Casler, 2012). Cultivars have undergone selection for agronomic traits or seed multiplication which both entail elimination of dormant seeds with potential for reducing cultivar dormancy (Casler, 2012). Trailblazer represents several seed accessions from Nebraska and Kansas that have potential intraspecific hybridization (Vogel et al., 1991). EG-2101 resulted from selection of 'Cave-in-Rock' for increased biomass production (Progressive Forage Grower, 2009; Christensen, 2010). Summer is the only cultivar in this study that has not been selectively bred but has undergone numerous generations of seed multiplication since its release as a cultivar (Alderson and Sharp, 1994). WS12L-IL consists of a broad genetic base of lowland germplasm selected for winter hardiness in Madison, WI, and represents a significant increase in yield than many other cultivars associated with its late flowering time (Casler, 2012).

Experiment 1: Test of Switchgrass Populations on Germinability and Dormancy

We tested the proportion germinable and dormant seeds of the cultivars Summer, EG-2101, WS12L-IL, Trailblazer, and eight wild populations (N = 12 populations) in a growth chamber between 28 Feb. and 15 Mar. 2013. Before the germination test, seeds were dry prechilled at 1 to 4°C for 10 mo and then surface sterilized with a 5% v/v bleach solution and triple rinsed with deionized water. For each population we established six replicate Petri dishes (100 by 15 mm) with moistened 6.1-mm Versa-Pak germination paper (Seedburo) each containing 50 fully formed, randomly selected seeds (six replicate Petri dishes by 50 seeds per replication = 300 seeds per population; $N = 72$ Petri dishes). We recorded dry mass (grams) of each 50-seed replicate. Petri dishes were randomly distributed in the growth chamber maintained at 30:15°C, 8:16 h day/night, respectively, for 14 d (Association of Official Seed Analysts [AOSA], 2010a). We recorded and removed germinated (radicle length ≥2 mm) seeds daily. After 14 d, we tested viability of nongerminated seed using a 0.1% tetrazolium solution (AOSA, 2010b). Germination proportion is calculated as the number germinated (g) divided by germinated (g) plus viable, nongerminated seeds (d): germination proportion = $[g/(g + d)] \times 100$. We removed seeds that were either confirmed dead by the tetrazolium test or covered by 90% mold, leaving 3307 seeds (91.9% of the original seeds) to estimate germination proportion.

Experiment 2: Test of Cold–Moist Stratification and Populations on Germinability and Dormancy

We selected three cultivars and five wild populations representative of the variation in germination observed in Experiment 1 and tested the effect of cold–moist stratification on germination from 9 Dec. 2013 to 24 Jan. 2014. Seeds were dry prechilled at 1 to 4°C for 19 mo before the start of Experiment 2 (9 mo after Experiment 1). We established five replicate Petri dishes each with 50 seeds per treatment (cold–moist and control) and population (five replicate Petri dishes per treatment by 50 seeds per replication = 250 seeds per population per treatment; $N = 80$ Petri dishes). We recorded dry mass (grams) of each 50-seed replicate. To impose the cold–moist stratification treatment, we randomly arranged all Petri dishes in a dark storage container at 4°C for 28 d. Cold–moist stratification seeds were surface sterilized with a 5% v/v bleach solution, triple rinsed with deionized water, and then placed on moistened 6.1-mm Versa-Pak germination paper. Control Petri dish seeds remained dry inside a coin envelope to prevent seed loss. After 28 d, Petri dishes were transferred to the growth chamber, control seeds were surface sterilized and moistened, and all Petri dishes were randomly arranged for the 14 d germination test using the same conditions as in Experiment 1.

Daily monitoring, data collection, tetrazolium tests, and germination calculations followed the protocols established in Experiment 1. We removed seeds that were either confirmed dead (negative tetrazolium test), covered by more than 90% mold, or showing damaged root growth, which left 3396 seeds (84.9% of the original seeds) to estimate germination proportion. Despite a longer saturation period for cold–moist stratified seeds, their mortality was only 2.3% greater than seeds in the control, and this difference was not statistically significant ($P = 0.35$).

Statistical Analysis

All data were analyzed in R version 3.1.1 (R Development Core Team, 2014). Petri dish was the experimental unit. We used the generalized linear mixed model glmer to conduct likelihood ratio tests (Chi-square distribution, χ^2) of the effect of switchgrass population (individual population, cultivar vs. wild), cold–moist stratification, and their interaction on germination in Experiments 1 and 2. The response of each seed followed a Bernoulli distribution (germinated, dormant) and Petri dish was treated as a random effect to account for nonindependence among seeds in the same Petri dish. Seed mass based on 50-seed samples was analyzed using ANOVA and model residuals were normally distributed and homogeneous among treatments. In Experiment 1, we evaluated all pairwise comparisons of germination and seed mass among each population using unadjusted P-values at the 0.05 level to provide a preliminary test of mean differences among populations. To address the potential for inflated experiment-wise type I error rate, we used the results from Experiment 1 to retest a subset of eight populations of interest (Saville, 2013). The effect of cold–moist stratification on each of the eight populations was evaluated using unadjusted P-values in Experiment 2.

Population-level analysis was performed with analysis of covariance on the mean seed mass and germination for each population in both experiments. In Experiment 1, we evaluated the effect of seed origin (cultivar vs. wild) and mean seed mass on germination at the population level. In Experiment 2, the population-level response to cold–moist stratification (germination with stratification minus germination without stratification) was evaluated in relation to mean seed mass, germination without stratification, and origin (cultivar vs. wild).

RESULTS AND DISCUSSION

Wild and cultivar populations of switchgrass were tested to evaluate differences in germination proportion and, from this, progress in breeding efforts for improved germination. We also tested the response of switchgrass populations to cold–moist stratification to determine the benefit of stratification treatments for further improving germination. Germination of the four cultivar populations significantly exceeded that of eight wild populations (57.1 vs. 18.5%; $\chi^2 = 43.1$, $P < 0.001$; Fig. 1a). There were also significant differences in germination within each group, especially among wild populations ($\chi^2 = 150.6$, $P < 0.001$; Fig. 1a). We then used the same procedure to retest germination in a subset of populations representative of the variation in germination. Consistent with Experiment 1, there were significant differences in germination among switchgrass populations ($\chi^2 = 117.3$, $P < 0.001$). The relative differences and mean statistical comparison results were generally consistent between experiments (Fig. 1a and control treatments of Fig. 2), further supporting the finding of large variation in germination among wild populations and generally higher germination of cultivars.

Higher seed dormancy of wild vs. cultivar switchgrass populations likely reflects different selection history. Dormancy may be reduced for cultivars by elimination of nongerminating seeds during successive cycles of selection for other agronomic traits or seed multiplication as mechanisms that unconsciously select against dormancy (Casler, 2012). The significant response of switchgrass dormancy to selection suggests there is a strong genetic

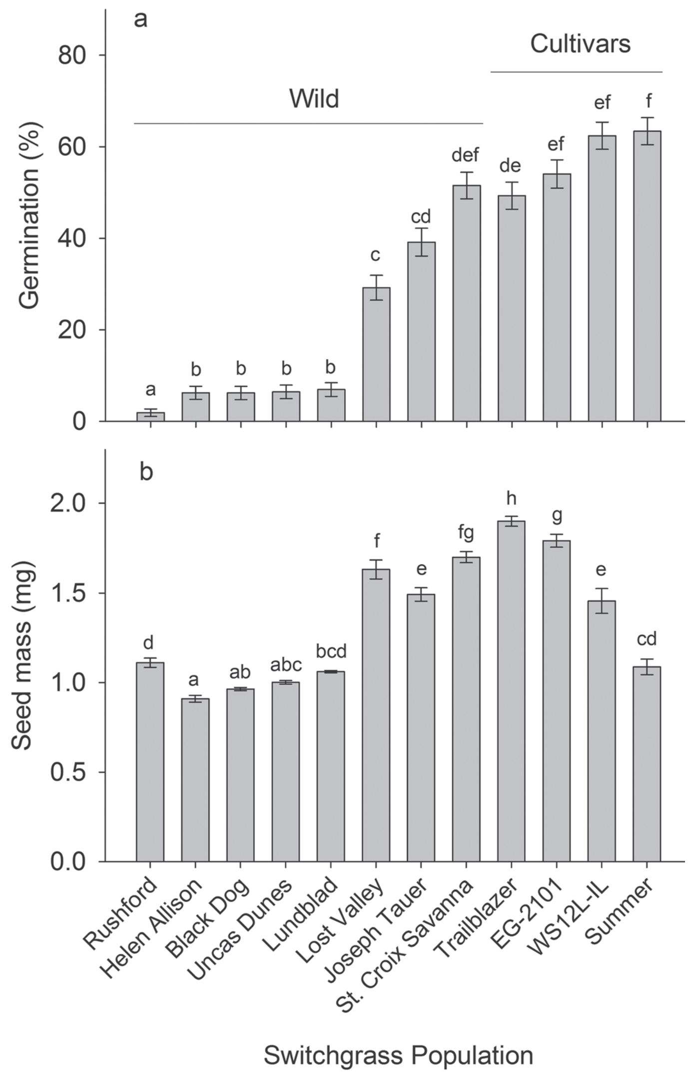

Figure 1. Mean (±SE) (a) germination and (b) seed mass (based on samples of 50 seeds) of eight wild and four cultivar populations of switchgrass. Letters indicate significant differences among populations ($P < 0.05$). The names of wild populations represent their collection sites.

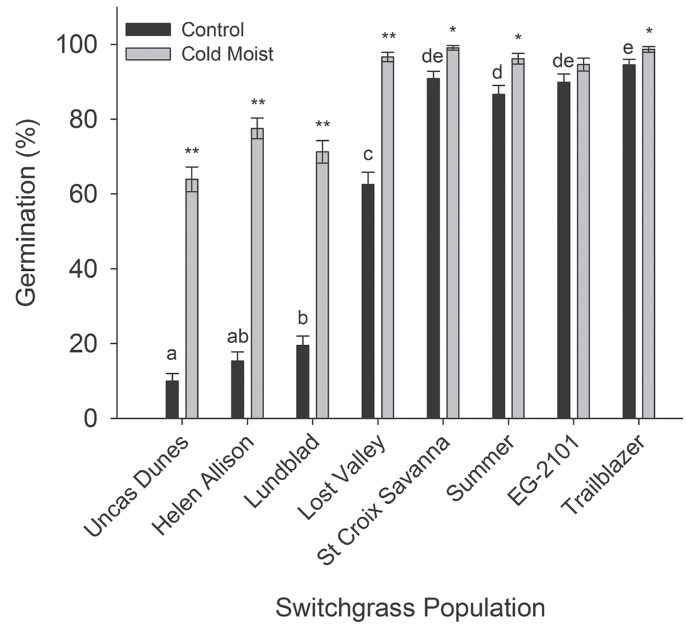

Figure 2. Mean (±SE) germination of switchgrass populations in relation to cold–moist stratification. Letters indicate significant differences ($P < 0.05$) among switchgrass populations without stratification. Asterisks indicate statistical significance of the effect of cold–moist stratification (**, $P < 0.001$; *, $P < 0.05$).

basis and high heritability for dormancy (Burson et al., 2009), as shown in other plants (Goggin et al., 2010). In contrast to cultivars, wild populations persist in environments with elevated spatiotemporal heterogeneity in growing conditions. Seed dormancy is adaptive in such environments, allowing for the formation of seedbanks that persist through times of unfavorable conditions (Pake and Venable, 1996; Rees et al., 2006). While longer-lived plants often show less seed dormancy than shorter-lived plants under variable environments (Rees, 1993; Rees et al., 2006), there is still considerable dormancy in perennial plants including switchgrass (Shen et al., 2001), as our results confirm. While most wild populations were highly dormant, several showed low dormancy. Reseeding wild populations may selectively reduce dormancy if recently disturbed restoration environments promote rapidly germinating seedlings over dormant seeds. There is some evidence in support of this hypothesis, as the only reseeded populations, Joseph Tauer and Lost Valley, showed low dormancy (Fig. 1a). Nevertheless, these results demonstrate genetic variation for seed dormancy levels in native switchgrass, establishing the basis for genetic improvements in germination of commercial cultivars.

Seeds of switchgrass cultivars were 26% heavier than wild switchgrass seed (1.56 vs. 1.23 mg seed^{-1}; $F_{1,70} = 16.4$, $P < 0.001$), but there was substantial variation in seed mass among wild and cultivar populations ($F_{11,60} = 100.76$, $P < 0.001$; Fig. 1b). Seed mass, origin (cultivar, wild), and their interaction explained most of the population-level variation in germination ($R^2 = 92.1\%$, $P < 0.001$, Fig. 3). There was a strong positive relationship between seed size and germination for wild populations ($P < 0.001$) but not cultivars ($P = 0.16$; Fig. 3). This pattern was confirmed with the subset of eight cultivar and wild populations retested in Experiment 2. Selection for low dormancy itself and selection for larger seed mass may jointly influence seed germination as the current study and others indicate (Vange et al., 2004). Greater seed size can provision resources for seedlings to endure variable establishment conditions, as experienced by wild populations in natural environments (Rees, 1993; Leishman et al., 2000), but seed size may be less important to cultivars where it has been shown to only have an ephemeral effect on seedling performance in more homogeneous and predictable agricultural environments (Smart and Moser, 1999). Alternatively, because cultivar seed was generally much larger than that of wild populations and limited in its range of sizes, the potential to

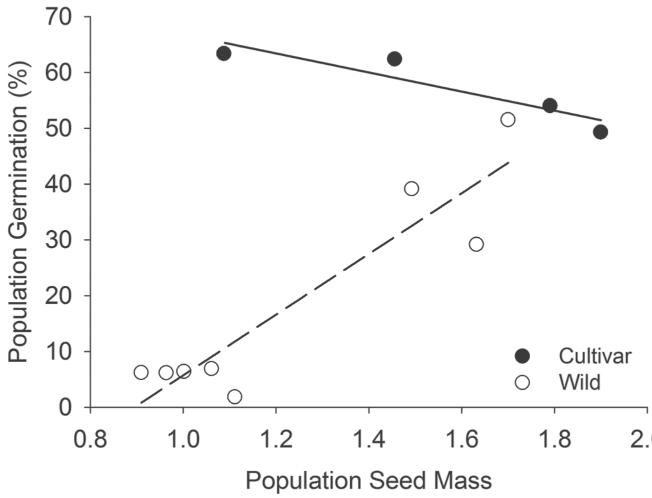

Figure 3. Mean seed mass and origin (cultivar, wild) vs. germination for eight wild and four cultivar populations of switchgrass.

detect a relationship between seed size and germination in cultivars may be lacking. Aiken and Springer (1995) showed a positive correlation between seed size and germination in cultivars up to approximately 1.0 to 1.2 mg $seed^{-1}$, at which point, further increases in seed size did not promote germination. The majority of our cultivars were larger than 1.2 mg $seed^{-1}$. Regardless of the underlying mechanism, these data suggest both seed size and selection history jointly influence germination.

Seed of all populations were grown in different environments, and some populations were subjected to different storage conditions and duration. While these environmental differences were, to some extent, unavoidable (i.e., wild populations must be collected from tallgrass prairies to be considered a wild population), they raise the potential for genotype × environment covariance to confound our inference on the role of selection history in germination. To minimize the potential for systematic environmental effects to confound our inference of genetically based differences among populations, we collected and compared numerous wild populations with cultivars with a known selection history. Our results are consistent with other studies suggesting a strong genetic basis for dormancy (Vange et al., 2004; Burson et al., 2009; Goggin et al., 2010). Further, in a study that directly compared both genotype and environmental effects, dormancy rankings among maternal genotypes were conserved across environment (Platenkamp and Shaw, 1993). Therefore, while growing environment may have influenced dormancy in our study, its effect is likely nonsystematic as to not alter the general patterns in germination among cultivar and wild populations. Further, our estimates of dormancy are relevant to growers and breeders seeking to incorporate wild populations and current cultivars into research and breeding projects.

Dormancy is also mediated by seed age and storage conditions (Zarnstorff et al., 1994). All seed was stored at 1 to 5°C for 10 mo before the first experiment began, but beforehand, two cultivars (EG-2101 and Trailblazer) were stored for 12 mo longer and at warmer temperatures than all other populations. Warm storage temperatures (23 to 30°C) for 3 to 12 mo can substantially enhance germination (Zarnstorff et al., 1994; Shen et al., 2001). However, our data suggest differences in storage conditions did not significantly contribute to population-level variation in germination in Experiment 1. For example, the two cultivars with extended storage times and exposure to warmer temperatures showed slightly lower germination than the other cultivars. From the first to second experiment we observed a 4 to 45% increase in germination when seeds were stored at 1 to 5°C for 1 yr more; highly dormant populations remained mostly dormant (Fig. 1a vs. 2). While we urge caution in interpretation of the effects of aging on germination because we did not experimentally test this effect (e.g., by controlling for observer effects of seed selection in each trial), these observations are consistent with the hypothesis that some wild populations of switchgrass are persistently dormant even after an extensive after-ripening period.

Cold–moist stratification treatment increased germination overall ($\chi^2 = 100.1$, $P < 0.001$) and for all but one population, EG-2101, which showed a consistent but nonsignificant trend ($P = 0.13$; Fig. 2). The magnitude of the treatment effect varied greatly among populations ($\chi^2 = 23.3$, $P = 0.001$; Fig. 2). This is consistent with other experiments that showed strong positive effects of cold–moist stratification on seed germination and emergence with large variation in the response among different cultivars and seed lots (Zarnstorff et al., 1994; Shen et al., 2001). We further evaluated variation in population-level response to stratification (population germination under cold–moist stratification minus germination of control) and tested variables associated with the response. There were no significant effects of seed mass ($t = -0.35$, $P = 0.74$) or origin ($t = 1.2$, $P = 0.31$) on response to cold–moist stratification. Instead, populations with a higher dormancy showed a greater response to cold–moist stratification ($t = -12.7$, $P < 0.001$), explaining 95.8% of the variation (R^2) in response (Fig. 4). For every 10% increase in dormancy among populations, there was a 6.5% increase in the response to cold–moist stratification, suggesting an important offsetting effect of this seed treatment. Other studies have similarly shown that cold–moist stratification increased the germination of highly dormant populations much more so than less dormant populations (Zarnstorff et al., 1994; Shen et al., 2001).

In conclusion, seed origin and mass jointly influenced population-level variation in switchgrass germination. This information can be used in breeding programs to improve switchgrass germination and establishment success as well as provide an initial roadmap for screening and integration of wild populations into current breeding programs.

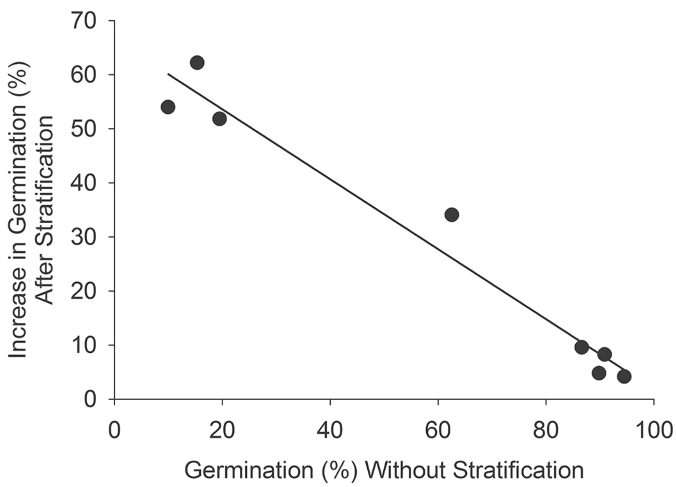

Figure 4. Relationship between mean germination of each switchgrass population and the increase in germination after cold–moist stratification (germination with stratification minus germination without stratification for each population).

This study has also shown that cold–moist stratification increased germination of nearly all populations, suggesting that such treatments could be widely applied to enhance germination in the field. Future studies could use seed grown and stored in similar environments to improve the estimation of population genetic variation in dormancy.

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