Improved Establishment Methods for Native Warm Season Grasses

A Research Study
Presented for the
Master of Science
Degree in Agricultural Operations Management
The University of Tennessee, Martin

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Fall 2008

INFORMATION

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Date of Degree: December 13, 2008

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Major Field: Agricultural Operations Management

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Title of Study: *Improved Establishment Methods for Native Warm Season Grasses*

Pages Contained in Study: 35

Candidate for: Master of Science

DEDICATION

This paper is dedicated to my late father who always demanded the best of me and stood behind me. He taught me more than books ever could. He taught me to serve the Lord, love my neighbor and work hard. Thank you for everything and I know you are in Heaven watching over me.

I would also like to dedicate this to a best friend that passed on way too soon, Levi Satterfield. Levi touched and cared for so many in his short life here on earth. No matter how hard my life gets I remember all that you fought through.

ACKNOWLEDGEMENTS

I would like to thank Dr. Bruce Greene at Tennessee Technological University for the opportunity to contribute to ongoing research and also for guidance and assistance even though I was not a current student. Without his help this research would never have been possible.

ABSTRACT

Native warm season grasses (NWSG) are becoming more important in the agricultural industry. Their ability to withstand drought and produce high yields on marginal soil and fertility make them valuable to livestock producers during these times of climatic change in the Southeastern United States. Switchgrass, a species of perennial warm season grass, is also being explored extensively as a biomass crop for ethanol production. For perennial warm season grasses to be more widely used, establishment methods and germination rates must be improved to allow for a harvestable stand in fewer than two growing seasons.

This study evaluated four practical establishment methods. Method 1 was the no-till establishment of NWSG following a spring cover crop with no herbicide treatment. Method 2 was the no-till establishment of NWSG following a glyphosate herbicide application 30 days prior to seeding. Method 3 was broadcast seeding of NWSG following a glyphosate herbicide application 30 days prior to seeding and conventional seedbed preparation immediately prior to seeding. Finally, Method 4 was broadcast seeding following conventional tillage 30 days prior to another tilling immediately prior to seeding. Three species of NWSG were used in the trial: (1) Pete, eastern gamagrass (*Tripsacum dactyloides* L.); (2) Alamo switchgrass (*Panicum virgatum* L); and (3) Rumsey indiangrass (*Sorghastrum nutans(*L.) Nash). The research was conducted at two locations in Tennessee: Putnam County (PC) and Trousdale County (TC). A split plot design was used with locations as blocks. Establishment methods were main plot treatments and species were subplot treatments.

The results indicated that establishment had no significant ($\alpha = 0.05$) effect on weed control or germination. Alamo switchgrass had germination rates significantly ($\alpha=0.05$) higher than Pete eastern gamagrass and Rumsey indiangrass.

TABLE OF CONTENTS

Page

LIST OF TABLES AND FIGURES

TABLES **Page**

FIGURES

Chapter 1

Introduction

Interest in native warm season grasses (NWSG) has grown in recent years as energy costs have increased and periods of drought have become more common in the southeastern United States. Once established NWSG withstand drought better than current forages, due to a deep root system. They are also very productive with limited inputs and can produce yields of 4.5 - 11.2 t ha^{-1} (2-5 tons ac^{-1}) (Harper et. al., 2007). These traits make NWSG attractive as a potential forage option in drought situations. Developing technologies in the biofuels industry has led to increased interest in energy from biomass crops. Most current biomass research has been conducted on switchgrass (*Panicum virgatum* L.). Two other important NWSG species are indiangrass (*Sorghastrum nutans* (L.) Nash), eastern gamagrass (*Tripsacum dactyloides* L.). These three species are valuable in forage production and wildlife habitat. Switchgrass has garnered most of the interest from the biofuel industry.

The importance of these grasses is increasing in the southeast U.S. due to the biofuel initiative, wildlife habitat enhancement, and warm season forage production. However, establishment of these grasses can be costly, labor intensive, and time consuming. All NWSG have problematic establishment as they are not as competitive as summer annual weeds and other warm season grasses. Therefore, weed control becomes critical in the establishment of these grasses. Most NWSG also require very careful seedbed preparation and/or special equipment to sow properly (Harper et. al., 2007).

Some NWSG also have problems with variability in germination. Seeds of eastern gamagrass are enclosed in a cupule. This cupule makes it difficult for eastern gamagrass to germinate and usually requires cold-moist stratification is required to achieve adequate germination results in eastern gamagrass.

Objectives

This study will evaluate the effect of four practical establishment methods on NWSG germination and weed control using three common NWSG: (1) Pete, eastern gamagrass; (2) Alamo, switchgrass; and (3) Rumsey, Indiangrass.

Chapter 2

Literature Review

Cool Season vs. Warm Season

Tennessee's forage industry is based primarily on cool season grasses that thrive in spring and fall but struggle to produce adequate forage in summer. These cool season grasses also lose quality in the summer. The growth curve for NWSG compliments cool season grasses (Figure1). NWSG can bridge the gap in the cool season growth curve more economically than other warm season grasses such as Bermudagrass and crabgrass, both of which require costly fertilizer and high levels of management to provide maximum yields (Bates, 2007).

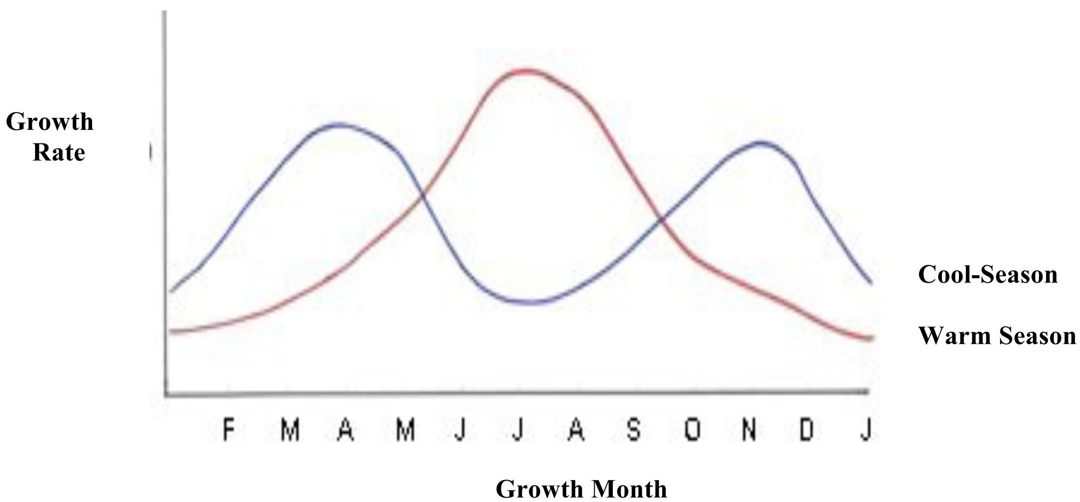

Figure 1. Growth curves of cool and warm season grasses
(Source: UC Davis 2004).

Native Warm Season Grasses

Switchgrass is a warm season perennial grass native to the United States. It is a deep rooted bunchgrass that grows up to 2.1m (7 ft) tall, producing large amounts of biomass (cellulose) that can be digested and converted to ethanol. This makes switchgrass an excellent choice for biomass energy production. With all the publicity about its biomass capabilities, switchgrass's forage potential is often overlooked (Harper and Keyser, 2008). Depending upon rainfall and management practices, switchgrass can yield from 4.5 - 13.5 t ha^{-1} (2 - 6 tons ac^{-1}). If harvested at the appropriate maturity, switchgrass has surprisingly good quality with 16 - 17% protein. However, switchgrass is a small seeded grass that is slow to establish.

Indiangrass is a warm season perennial grass native to tall grass prairie of the U.S. It is a deep-rooted bunchgrass that grows 0.9 - 1.8m (3 - 6 ft.) tall and used primarily as pasture. Indiangrass has higher quality than most other warm season perennial grasses (Ball et. al., 2002). It can be expected to produce from 4.5 – 10.1 t ha^{-1} (2 - 4.5 tons ac^{-1}) with average rainfall. Under better conditions and adequate management, higher yields can be expected. Indiangrass has a very light fluffy seed that makes it difficult to sow. When seeded it grows slowly and competes poorly with weeds.

Eastern gamagrass is warm season perennial bunchgrass that is native to Eastern U.S. and Eastern Great Plains. Some varieties form large bunches that may make it unacceptable as a hay crop. It is difficult to establish and it can be difficult to break seed dormancy. Eastern gamagrass has yields comparable to switchgrass but difficulties in establishment typically limit its use (Harper et. al. 2007).

Biomass

Many biofuel studies have been conducted to examine the conversion of biomass products into biofuels. Switchgrass could be a major source of biomass. Lowland switchgrass varieties, such as Alamo, are well suited to Tennessee and are very productive, making them attractive as biomass feedstock. In tests, these lowland varieties have produced yields up to 22.5 t ha^{-1} (10 tons ac^{-1}) (Garland, 2007). On a commercial scale a more realistic 13.5 - 18 t ha^{-1} (6-8 tons ac^{-1}) can be expected. This amount of dry matter can be turned into an estimated 1,892 L (500 gal) of ethanol. It takes about three years for a stand of switchgrass to reach its maximum production level. Therefore, plant establishment is critical. Once established a well managed stand of switchgrass can remain productive for 10-20 years (Garland, 2007).

Germination

Seed germination is the major obstacle hindering the use of NWSG, which typically have low germination rates. Seeds of eastern gamagrass are enclosed in cupules and germination rarely exceeds 60% (Klein et. al., 2007). There have been many studies to evaluate germination based on seed size, seed color, and other characteristics that may indicate seeds with higher germination rates. Aiken and Springer (1995) and Smart and Moser (1999) evaluated switchgrass seed size and its affect on germination and seedling growth. The results showed that heavier seeds germinated at higher rates than lighter seeds. These studies also showed that after 8 - 10 weeks there was little difference in seedling development between heavy and light seed.

Switchgrass is one species in which seed size may indicate its germination potential. Smart and Moser (1999) studied the effects of seed size on germination, seedling growth, and development at the University of Nebraska. The study evaluated two seedlots, from each of two cultivars (Blackwell and Trailblazer). Seeds were separated by weight. Heavier seeds had higher germination rates than lighter seeds, confirming the findings of previous studies. The study also revealed that after 8 - 10 weeks, the seedlings from lighter seeds were the same size as seedlings from the heavier seeds. The researchers concluded that seed weight affects germination but has minimal long-term effects on seedling growth and development (Smart and Moser 1999).

Hanson and Johnson, (2005) evaluated switchgrass seed germination in response to pH and temperature. Eight switchgrass cultivars, five temperatures (5 - 40ºC), and nine pH levels (pH 4 - 10) were used in the study. The optimum temperature for germination was 25ºC. No seeds germinated at 5ºC. The average germination rate at the optimum pH of 6 was 38.3%. On average, first day of germination was day 7.3. Switchgrass performed well in pH 5 – 8, but performance dropped sharply below pH of 4.6 and above pH of 8.0 (Hanson and Johnson, 2005).

Eastern gamagrass exhibits seed dormancy that is overcome by stratification. (Roberts and Kallenbach, 1999). Studies have been done to study how the cupule, which encloses the seed, affects seed germination. Klein et. al. (2007) evaluated cupule color and how well it indicated germination rates of seeds treated by stratification and hydrogen peroxide treatments. The seeds were sorted into three color groups; green, medium (brown-green), and dark (brown) categories. The seeds were stratified for six weeks at 4ºC or were subjected to one of five hydrogen peroxide treatments for 18 h. In the absence of stratification or hydrogen peroxide treatments, no seeds germinated after 14 days. The dark cupules generally had better germination and vigor than the green cupules in both stratified and hydrogen peroxide treated seed. Therefore, color sorting seeds improved eastern gamagrass seedling germination and vigor. In addition, soaking seeds in 15% hydrogen peroxide for 18 h substituted for the standard six-week stratification normally used to break dormancy (Klein et. al., 2007).

Establishment

Establishment methods for NWSG vary in time and labor required. Several planting methods have been evaluated for their effectiveness on improving germination and stand establishment in NWSG. Many factors, including rainfall, temperature, weed competition, and planting date determine the effectiveness of the establishment practices. Weed competition in NWSG stands used for forage and biomass production are a detriment to a successful stand. However, for wildlife plantings certain species of weeds enhance the benefit of the area to wildlife.

A two year study by Aberle et. al. (2003) at Iowa State University evaluated the effects of planting date, planting depth, and seed treatment on establishment of eastern gamagrass. A seedling survival rate of 26% was considered to be successful for this trial. The following methods met these requirements: unstratified seeds planted on Aug. 16, stratified seed planted at both depths on Nov. 1, stratified seed planted on April 14, unstratified seed planted at both depths and stratified seed planted at 2.5 cm on June 16. Normal recommendations for seeding eastern gamagrass in the Corn Belt were from November to February. This study suggested that mid-August through late October/early November is also an adequate time to plant eastern gamagrass (Aberle et. al., 2003).

Switchgrass has received considerable research interest in recent years because of the push for biofuel production. A 2002 Mississippi State University study evaluated eight establishment systems (4 Fall Plantings, 3 Spring Plantings, and 1 Summer Planting). Alamo switchgrass was broadcast or no-till seeded at 6.72 Kg ha $^{-1}$ live seed (6 lb ac $^{-1}$ live seed). Gramoxone (0.45 Kg [1 lb] a.i.) and Roundup-Ultra (0.45 Kg [1 lb] a.i.) were used for weed control. Gramoxone on April 1, followed by preparing a clean tilled seedbed and broadcast planting in mid-April resulted in a 75% stand of switchgrass. No other treatments resulted in success. In the following year, the only successful treatment was the same Gramoxone treatment (Edwards, 2002). A separate study using switchgrass, big bluestem, and Indiangrass gave similar results as the study conducted the previous two years (Edwards, 2002).

Very few herbicides are labeled for pre-emergent or post-emergent grass weed control in NWSG. A University of Tennessee study evaluated Plateau herbicide on conventional and no-tilled NWSG establishment at four experiment stations across Tennessee. Plot preparation prior to seeding varied with location. Four applications of Plateau were evaluated for weed control: 8oz pre and post-emergence, and 12oz pre and post-emergence. The researchers concluded that NWSG can be established successfully by using either conventional tillage with top-sowing or no-till drilling if adequate moisture is present. Pre-emergence Plateau applications provided better weed control than post-emergence applications. Seeding rates should also be matched to management objectives (Harper et. al., 2002).

Chapter 3

Materials and Methods

This study was conducted in late spring at two locations: Putnam County (PC), TN and Trousdale County (TC), TN. The PC study site was in a flood plain along a small creek with *Lindside* silt soils and the TC study site was on upland terrain with *Mimosa-Rock* outcrop complex and 20 – 45 % slope. The previous history for each site was recorded because it may affect how the establishment methods were able to control weeds. Previously, the TC site was a tall fescue hay field that had chemical weed control applied in past years to control broadleaf weeds. The PC site had been a field overgrown with many species of summer and winter annual and perennial weeds. There had been no weed control methods used at the PC site prior to this experiment.

There were three species of NWSG evaluated in this study: (1) Pete, eastern gamagrass; (2) Alamo, switchgrass; and (3) Rumsey, Indiangrass. Alamo switchgrass is a low-land cultivar. It is late maturing and its leaves are coarser than those of other varieties of switchgrass. Rumsey indiangrass is relatively late to mature but displays rapid growth in mid to late summer. Pete eastern gamagrass is a superior seed producer (Harper et. al., 2007).

Establishment Treatments

Four establishment treatments were used in this study with three species and two repetitions at two locations.

1. Establishment Method 1 (M1) – Establish a spring oat cover crop in late winter; harvest the crop as a hay in May followed by no-till establishment of the NWSG into existing sod.

2. Establishment Method 2 (M2) – Glyphosate herbicide application (2qts ac^{-1}) one month prior to seeding followed by no-till establishment of NWSG into existing sod.

3. Establishment Method 3 (M3) - Glyphosate herbicide application (2qts ac^{-1}) one month prior to sowing followed by conventional cultivation immediately before broadcast seeding the NWSG; this was followed by culti-packing to cover the seed no more than ¼ inch and to pack the soil.

4. Establishment Method 4 (M4) - Conventional cultivation one month prior to seeding and again immediately before broadcast seeding, followed by culti-packing to cover the seed no more than ¼ inch and to pack the soil.

Experimental Design

A split plot design with establishment treatments would as main plot and species as subplots. The species were randomly assigned to each row of subplots. The layout for each location is shown in Table 1.

There were 2 blocks with 4 main plots and 24 subplots, three observations made at various dates. Germination rates for these grasses were determined in a prior experiment in a greenhouse germination test. The germination rates for each species of NWSG ranged from 45-50% (Table 2).

The University of Tennessee recommends seeding rates of 8.97 – 11.21 Kg live seed ha^{-1} (8 - 10 lbs live seed ac^{-1}) for eastern gamagrass and iIndiangrass and 6.72 – 8.97 Kg live seed ha^{-1} (6 - 8 lbs live seed ac^{-1}) for switchgrass. Because of the late seeding date of this study, 13.45 Kg live seed ha^{-1} (12 lbs live seed ac^{-1}) was used for all species due to the possibility of dry weather following seeding.

Table 1. Plot Design

Trousdale County (Block 1)							
Method 1		Method 2		Method 3		Method 4	
Main	Plot	Main	Plot	Main	Plot	Main	Plot
Subplots		Subplots		Subplots		Subplots	
Indian	Indian	Eastern	Eastern	Eastern	Indian	Switch	Eastern
Switch	Eastern	Indian	Switch	Switch	Switch	Indian	Indian
Eastern	Switch	Switch	Indian	Indian	Eastern	Eastern	Switch

Putnam County (Block 2)							
Method 1		Method 2		Method 3		Method 4	
Subplots		Subplots		Subplots		Subplots	
Switch	Indian	Indian	Switch	Eastern	Indian	Switch	Indian
Eastern	Switch	Eastern	Eastern	Indian	Switch	Indian	Eastern
Indian	Eastern	Switch	Indian	Switch	Eastern	Eastern	Switch

Table 2. Germination rates and seed purity for each NWSG used in this experiment

NWSG	Germ. Rate	Pure Seed	PLS
Switchgrass	47%	99.8%	46.9%
Indiangrass	45%	85.4%	38.4%
Eastern gamagrass	50%	99%	49.5%

Experimental Procedures

M1 plots were sprayed on March 4 with 4.68 L ha^{-1} (2 qt ac^{-1}) of a glyphosate herbicide. A 95 L (25 gallon) ATV sprayer calibrated at 65.31 L ha^{-1} (6 gal ac^{-1}) was used to deliver the accurate amount of product to the plots. Two weeks following the glyphosate application an oat cover crop was drilled into the plot at a rate of 107.6 kg ha^{-1} (3 bushels ac^{-1}). The cover crop was harvested as hay on May 14. The NWSG were established on June 6.

M2 and M3 plots were sprayed on April 19, 2008. The NWSG were to be seeded one month after the glyphosate applications but due to rain, plots were not seeded until June 6.

M4 plots were tilled on April 23, 2008. Immediately before seeding on June 6, 2008, M3 and M4 plots were tilled to prepare the seedbed. Following seeding they were culti-packed.

Each subplot was a 3.05 m x 6.10 m (10 x 20 ft) plot. The 3.05 m (10 ft) plot width worked well with the width of the equipment. There was a 1.52 m (5 ft) buffer around each plot. The drill used to seed the M1 and M2 plots was a 1.82 m (6 ft) Truax Flex II Series Grass Drill. The Truax drill had three seed boxes: one for small seed, a fluffy grass seed box and a large seed box. The drill was calibrated to deliver half the rate of seed per unit area. Two passes was required to cover the entire plot. To ensure no seeds were drilled into the wrong plot the remaining seeds were vacuumed out after seeding. Cultivation for the M3 and M4 plots was done with a 3.05 m (10 ft) disc to initially breakup the sod. Then a roto-tiller was used to prepare the seedbed. The broadcast seed was spread by hand to ensure an even seed cover. A filler of cat litter was added to the small switchgrass seeds so that they could be spread evenly. After the broadcast seeding, a Brillion cultipacker was used to pack the soil.

Data Collection and Analysis

After plot establishment, observations were made once adequate moisture was available for germination. Due to the lack of moisture during the month of June (Table 3) the observations began on July 2, 2008. Date was also collected on July 14 and July 21. Percent ground cover and estimated germination rate was determined for each plot. Observations were made until ground cover would no longer allow further seed germination. Weed pressure was estimated using percent ground cover. Germination rate of each NWSG was also estimated. A 100 cell grid plastic sheet was used to estimate weed cover. The number of cells containing weed growth was used as the percentage of ground cover. At least 10 observations were taken in each plot and average ground cover was recorded. Average germination rate for each plot was estimated by assigning a value between 0 and 5. Due to weed pressure from competing grasses, it was not feasible to determine the actual germination rate for each plot. The overall germination rate for this study was very low due to lack of rainfall following seeding (Table 3).

All data collected from this experiment were subjected to an ANOVA test using the SAS system. The means for each treatment were separated, with Student Neuman Keuls test for mean separations.

Table 3. Monthly rainfall for 2008 at locations (Lebanon, TN and Cookeville, TN) near the two sites.

Rainfall for Lebanon N 7 (nearest TC)		Rainfall for Cookeville	
Month	**Inches**	**Month**	**Inches**
April	6.41	April	5
May	No data	May	3.71
June	1.16	June*	3.9
July	4.11	July	4.62

* 75% of rainfall was before seeding or immediately prior to first observation
Compiled from NOAA Monthly Climate Data , NOAA Website.

Chapter 4

Results

Data analysis showed that establishment method had no significant ($\alpha = 0.05$) effect on ground cover or germination. Although the differences in ground cover percentage were not statistically different, the means for M2 (22%) and M3(25%) were lower than M1 (68%) and M4 (38%). M2 and M3 methods involved using glyphosate applications prior to establishment. Glyphosate applications proved to be better at controlling weed pressure over a longer period of time. M1 had more ground coverage at both test locations because the cover crops never completely died. The ground cover at seeding for M1 was higher than any other method (M2, M3, or M4) at seeding.

Percent ground cover at the PC location was greater than the ground cover at the TC site. The difference may have been due to the crop history of the two locations. Previously, the TC site was a tall fescue hay field. Tall fescue may have controlled warm season annual weeds in years prior to this experiment. The lack of weed growth in previous years reduced the number of weed seeds. This limited seed bank may have resulted in the lower ground cover data at the TC location (Figure 2). The late cultivation treatments at the TC location provided better control than at the PC location. Cultivation at the PC location opened the plentiful seed bank and allowed more seeds to germinate. Only the M2 method (glyphosate plus no-till) resulted in more ground cover at TC than PC. The presence of crabgrass (*Digitaria sanuinalis*) was much higher in M2 plots.

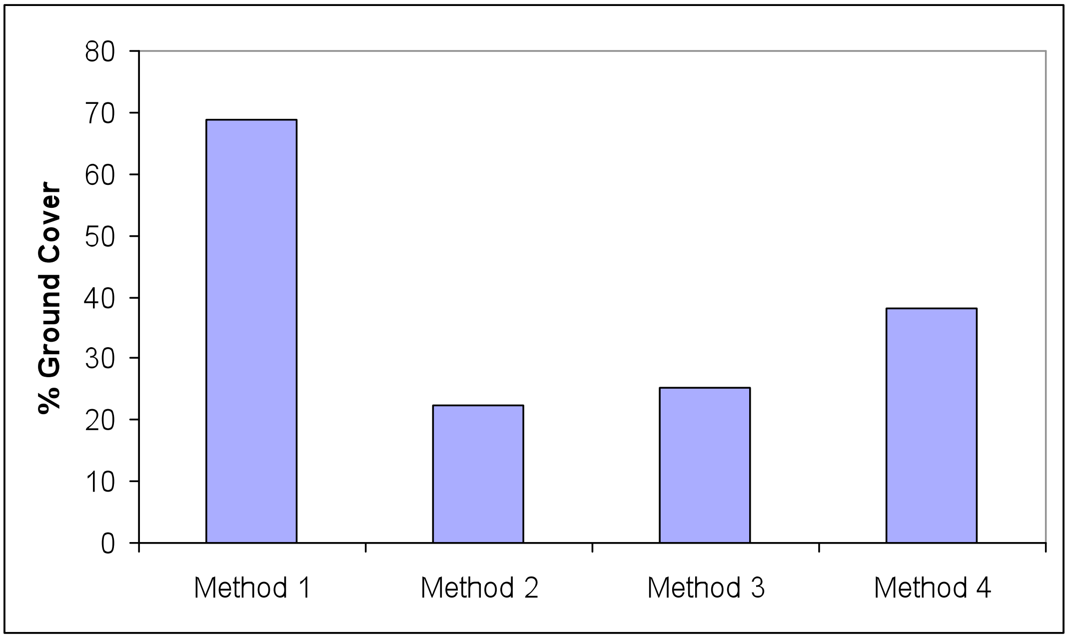

Figure 2. Effect of establishment method on ground cover
M1=cover crop; M2=glyphosate plus no-till; M3=glyphosate plus tillage; M4=tillage
(no significant differences among methods; $\alpha = 0.05$)

Analysis showed that method did not significantly($\alpha = 0.05$) affect germination. M2 had higher germination than the other methods followed by M3, M4 and M1 (Figure 4).

Germination rates among species were significantly different ($\alpha = 0.05$). Switchgrass germination was significantly higher than the other two species (Figure 5). Switchgrass was the only species that had any plot germinate greater than 25%. There was no statistical difference between germination of indiangrass and eastern gamagrass.

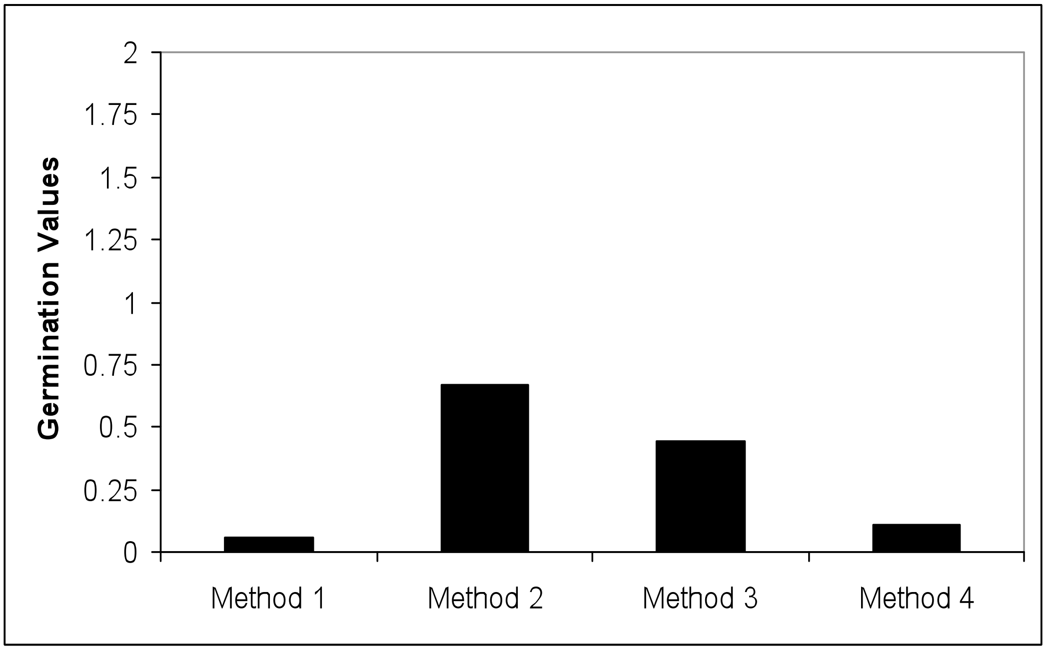

Figure 3. Effect of establishment on average germination rates.
Germ. Values-0=no. germ.; 1=1-10%; 2=11-25%
M1=cover crop; M2=glyphosate plus no-till; M3=glyphosate plus tillage; M4=tillage (no significant differences among methods; $\alpha = 0.05$)

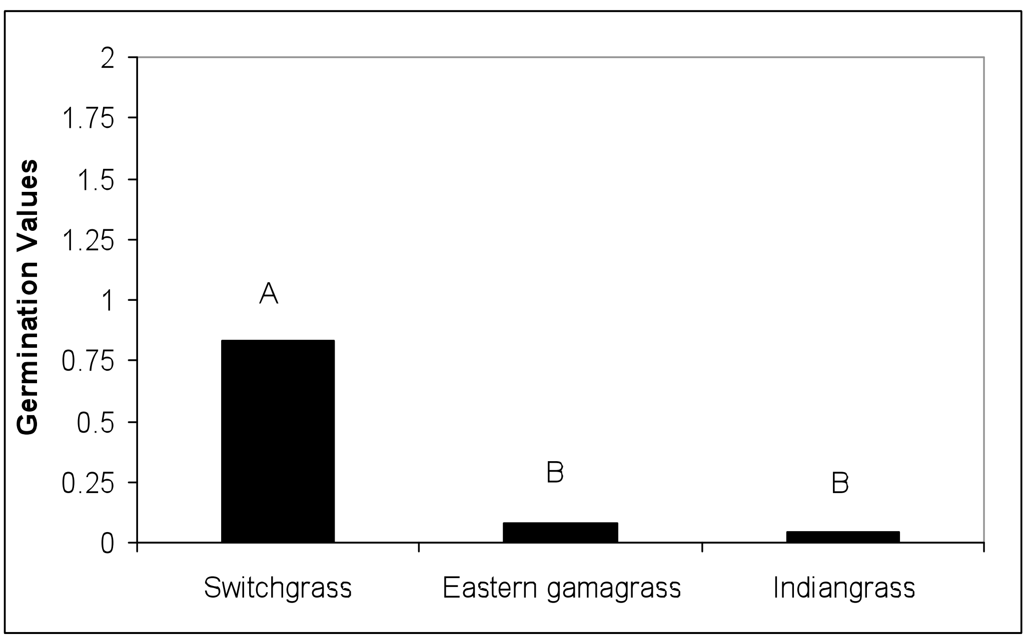

Figure 4. Effect of species on average germination rates.
Germ. Values-0=no. germ.; 1=1-10%; 2=11-25% . Species with different letters are significantly different by Student-Newman-Keuls test ($\alpha = 0.05$).

When observation date was added in the ANOVA analysis, date had a significant ($\alpha = 0.05$) effect on ground cover and germination rate (Figures 5 and 6). As the summer progressed the weeds continued to grow leading to the observed ground cover over time (Figure 5). There was a significant ($\alpha = 0.05$) difference in germination between the July 2 observation and the other observation dates (July 14 and July 21; Figure 6). The lack of rainfall prevented seed germination before July 2, so very few seedlings were observed until July 14.

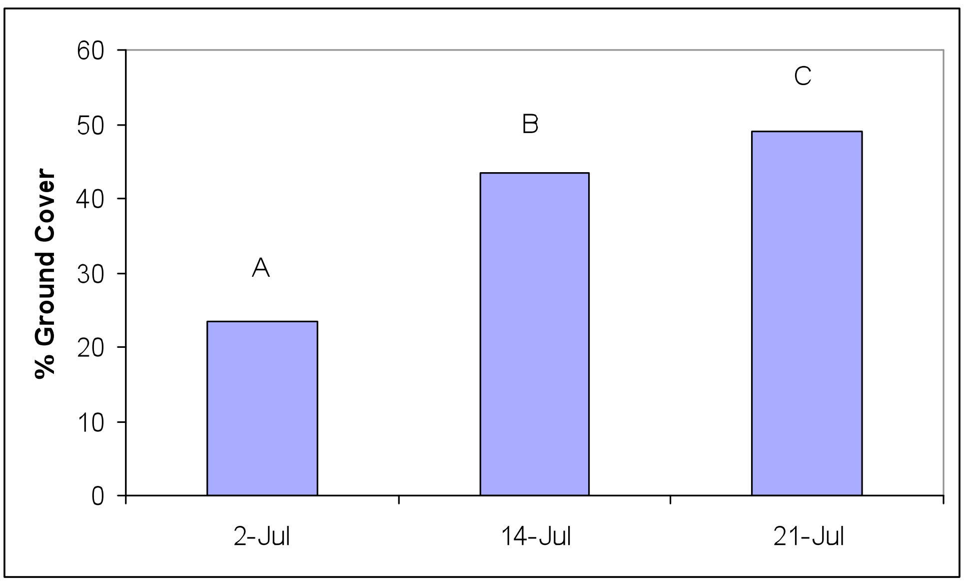

Figure 5. Effect of observation date on ground cover. Observations with different letters are significantly different by Student-Newman-Keuls test ($\alpha = 0.05$).

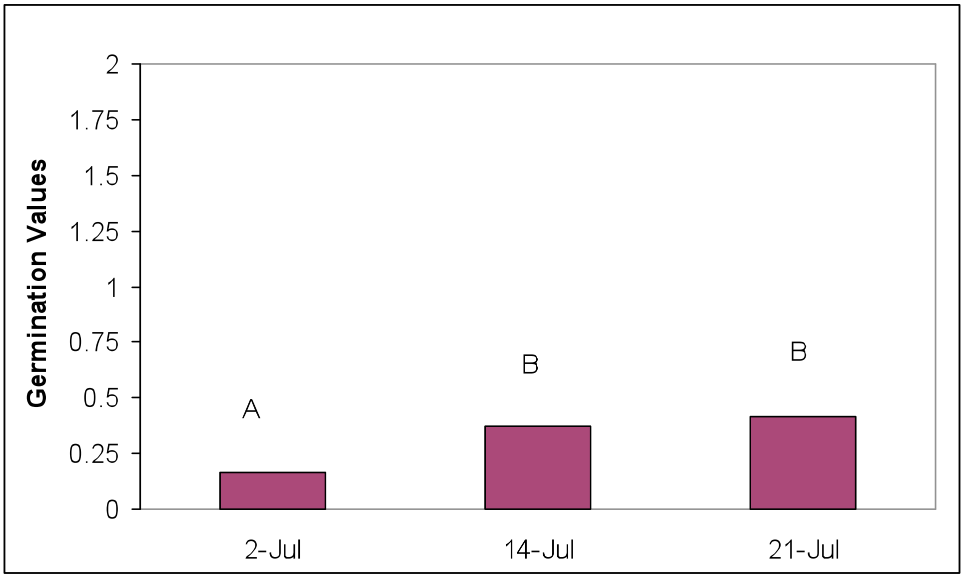

Figure 6. Effect of observation date on germination rates. Means with different letters are significantly different by Student-Newman-Keuls test ($\alpha = 0.05$).

Chapter 5

Conclusion

The results of this study were inconclusive due to the lack of rainfall and low germination rates. The study indicated that a glyphosate application at 2qts ac^{-1} had some effect on controlling weeds in no-till or conventional tillage top-sown applications, but differences were not statistically significant ($\alpha = 0.05$). No-till seeding may be more effective than top-sown seeding in limited rainfall situations. The study suggests it may be beneficial to establish NWSG using no-till methods in sites where weeds are prominent.

The present study did show that under limited rainfall situations, switchgrass had better germination rates than indiangrass or eastern gamagrass. The results indicate that more research is needed to evaluate establishment method. More replications of the establishment methods over several years would aid in delineating their effect on weed control and germination rate in NWSG.

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Appendix

Table A1. ANOVA for effect of method and species on ground cover.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr > F
Location	1	11130.25	11130.3	40.36	<.0001
Method	3	48603.5	16201.2	3.31	<.1757
Location*Method	3	14668.47	4889.49	17.73	<.0001
Grass	2	190.79	95.4	0.35	0.7082
Method*Grass	6	640.71	106.78	0.39	0.8861

Table A2. ANOVA for effect of method and species on germination rate.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr > F
Location	1	0.2500	0.250	1.50	<.0001
Method	3	8.9722	2.990	6.33	<.0819
Location*Method	3	1.4167	0.477	2.83	<.0001
Grass	2	19.055	9.527	57.17	0.7082
Method*Grass	6	20.277	3.379	20.28	0.8861

Table A3. ANOVA for effect of observation date on ground cover.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr > F
Location	1	11130.25	11130.3	77.88	<.0001
Method	3	48603.5	16201.2	113.36	<.0001
Location*Method	3	14668.47	4889.49	34.21	<.0001
Grass	2	190.79	95.4	0.67	0.5148
Method*Grass	6	640.71	106.78	0.75	0.6127
Time	2	17292.88	8646.44	60.5	0.0001

Table A4. ANOVA for effect of observation date on germination rate.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr > F
Location	1	0.25	0.25	1.61	0.2074
Method	3	8.972	2.991	19.22	0.0001
Location*Method	3	1.416	0.472	3.03	0.0317
Grass	2	19.055	9.527	61.22	0.0001
Method*Grass	6	20.277	3.379	21.71	0.0001
Time	2	1.722	0.861	5.53	0.005