Native Grasses: Improving the Seedling Vigor and Seed Production of Blue Grama (*Bouteloua gracilis*) and Prairie Junegrass (*Koeleria macrantha*) Ecovars™

BY GLENN MARK A. FRIESEN

A Thesis submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

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Winnipeg, Manitoba

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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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GENERAL ABSTRACT

Interest continues to grow in the utilization of native grasses for conservation, reclamation, Conservation Reserve Programs (CRP), right-of-ways, and wildlife habitat across North America. However, difficulty in establishment and limited availability of adapted seed sources has constrained the use of native grasses.

The objectives of this study were to assess the effects of seeding rate, phosphorous fertilizer, *Penicillium bilaii* and soil texture on the establishment of blue grama and prairie junegrass ecovars™, to examine the morphological distinctness and uniformity of a Manitoba blue grama ecovar™, and to determine the potential for protection of this ecovar™ under the Plant Breeders' Rights Act of Canada.

Blue grama and prairie junegrass row densities increased when seeding rate was doubled in a controlled environment; however seedling establishment as a percentage of seed sown decreased. Neither species responded to in-furrow P fertilizer, fungal inoculant treatment, or a liquid foliar application of N. Soil type was the most important treatment for increasing establishment success, with the sandy loam providing the highest establishment rates and largest plants for both blue grama and prairie junegrass.

The potential for the Manitoba blue grama ecovar™ to qualify for protection under the PBR Act of Canada was assessed as good. The ecovar™ was determined to be distinct from all pre-existing commercial populations included in this study (Bad River cultivar and the Wyoming and Minnesota ecotypes) over

both years of production. Additionally, the ecovar™ maintained levels of within-population variability equal to its original parental population and the Wyoming and Minnesota ecotypes, and showed more variability than the Bad River cultivar.

In conclusion, there is potential to improve the establishment success of native grasses by choosing the appropriate soil type. Furthermore, the possibility of protecting ecovars™ under the PBR Act of Canada should increase the availability of native grass seed sources in the future.

ACKNOWLEDGEMENTS

I wish to thank the following people whose assistance and support have made the completion of this project possible.

A sincere thanks goes to my previous advisor, Dr. S. Ray Smith for providing me with the opportunity to work on a project that combines my love for the environment and the forage industry with a hands-on approach to research. I am thankful for his encouragement, helpful suggestions, and patience during this project.

A special thanks to my new advisor, Dr. Rachael Scarth for making room for another graduate student in a demanding schedule. I am thankful for her insightful comments and for teaching me to pay attention to detail.

I would also like to thank Dr. Anh T. Phan for his guidance during the statistical analysis, and his ability to always make time for a friend in need. I'm especially appreciative of the numerous occasions we discussed population genetics and statistical analyses over a Tim Hortons coffee.

Thanks to the remaining members of my examining committee, Dr. Rene Van Acker for his encouragement and "open-door" policy, and Dr. Bruce Ford for his insightful comments.

I would also like to thank Anthony Mintenko for his sensible advice, Lyndsey Smith for her invaluable assistance during my field studies, and the entire summer staff for making our field work as much fun as possible.

Thanks also to friends and family for their constant support and encouragement. Special thanks to Orla, for her love and patience, and to my mom, for always having faith in me.

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1. INTRODUCTION

Native grasses, defined as those grass species indigenous to North America prior to European settlement, were the predominant vegetation in central North America and formed the tall grass, mixed grass and short grass prairie regions. Settlement led to the loss of large areas of native grassland through cultivation for annual crops, and through overgrazing. European and Eurasian forage species were planted on native pastures to improve livestock production (Smoliak and Slen, 1974), and on marginal cultivated lands for revegetation (Biondini and Redente, 1986). These introduced species were planted because they were often more vigorous and produced higher short term yields, and often out-competed existing native grass vegetation (Smoliak and Slen, 1974; Gerling et al.,1996). The suppression of natural prairie fires further reduced the competitiveness of remaining native grass communities (Stoddart et al., 1955; Leach and Givnish, 1996). Currently, only 1 per cent of the tall grass prairie and 20 per cent of the mixed grass prairie remain in Canada (Smith and Smith, 1999).

The 1930's witnessed the beginning of native grass revegetation in the Great Plains, when successive droughts, over-cultivation and wind and water erosion led to widespread crop failures and abandoning of farmland. Some estimates state that the abandoned farmlands would have required 50 years to return to their natural state without human intervention (Savage, 1934; Vallentine, 1971; Dormaar and Smoliak, 1985). Since there was a large land base that required reclamation and revegetation, the demand for native seed sources dramatically increased. However, seed harvests from remnant native stands were variable and infrequent, rarely occurring in the same area two years in succession (Kneebone, 1957). Additionally, problems of low native seed viability, high levels of seed dormancy, and poor seedling vigor limited the establishment of native seed production fields (Brown, 1943; Kneebone, 1957; Rathcke and Lacey, 1985). Therefore, by the 1940's and 1950's most revegetation efforts focused on introduced species like tall fescue (*Festuca arundinacea* Schreb.), smooth brome grass (*Bromus inermis* Leyss.) and crested wheatgrass (*Agropyron cristatum* L.), which establish easily and produce high seed yields (Jacobsen et al., 1994).

Studies during the last 30 years have shown that grass stands of many introduced species require regular replanting and maintenance (Duebbert et al., 1981). The relative uniformity of introduced grass stands has also led to the widespread loss in prairie and wildlife biodiversity (Wilson and Belcher, 1989). Consequently, there has been a renewed interest in the use of native grasses for reestablishment across the Great Plains. In the 1970's the USDA-Soil Conservation Service (now the Natural Resources Conservation Service, NRCS) re-focused their expertise on the collection and seed production of native grasses. Their efforts in cultivar development combined with the efforts of other public and private conservation groups have allowed the successful re-establishment of native grasses in North America.

Currently, native grasses are primarily used for soil conservation, prairie and park restoration, wildlife habitat conservation, highway and pipeline right-of-ways, mine reclamation (Jacobsen et al., 1994) and ornamental plantings (Davidson and Gobin, 1998). There is increased interest in native grasses for pasture and hay stands because of their forage production in the summer and fall and their stockpiling potential during the winter (Jefferson et al., 2000). However, their use continues to be limited by shortages of seed supplies. Conservation and reclamation projects have the largest requirement for native species, and are most affected by limited seed supplies. In many cases, reclamation projects are forced to use native grass seed produced from ecologically dissimilar regions. Movement of seed lots from their region of adaptation often leads to unsuccessful stand establishment (Kilcher and Looman, 1983). Reclamationists also debate the use of native grass cultivars because their uniformity may have negative effects on the bio-diversity of the surrounding ecosystem (Wilson and Belcher, 1989). Therefore the use of locally collected seed sources is often recommended (Smith and Whalley, 2002).

Limited supplies of locally collected seed combined with the need for regionally adapted plant material resulted in the development of a new type of seed source called ecological varieties, or ecovars™. Ecovars™ were first suggested by Erling T. Jacobsen of the United States Department of Agriculture – Natural Resources Conservation Service to combine genetic diversity with improved plant characteristics (e.g. seed production and seedling vigor). This development strategy is designed to improve stand establishment and seed production, while maintaining and/or improving plant and wildlife biodiversity. They have been defined as an intermediate step between a local native plant collection and a cultivar (Jacobsen, 1984). Ecovars™ differ from cultivars in that cultivars are typically selected to increase uniformity for ease of harvest, thus reducing genetic diversity. Currently, it has not been determined if the genetic diversity within ecovars™ will allow plant breeders to protect their investment given the strict guidelines of the Plant Breeders' Rights Act. The current parameters for Plant Breeders' Rights (PBR) require that new plant material be distinct from all other commercially available cultivars, uniform within their own stand, and genetically stable in successive generations (Canadian Food Inspection Agency, 2002).

Today, the native grass industry has expanded into many applications including: 1) urban landscaping, 2) right-of-way plantings to suppress weeds, reduce mowing and herbicide use, 3) revegetating after fire to prevent soil erosion, 4) re-establishing native grasses in nature preserves and parks in efforts to enhance biodiversity, and 5) seeding into pastures and hayland to increase forage quality, yield and duration of the grazing season.

The objectives of this study were to assess the effects of seeding rate, phosphorus fertilizer, *Penicillium bilaii*, and soil texture on the establishment of blue grama [*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths] and prairie junegrass [*Koeleria macrantha* (Ledeb.) J.A. Schultes] ecovars™ and to determine the distinctness and uniformity of blue grama cultivars, ecotypes and ecovars™ for the purpose of assessing the potential of the ecovar™ to qualify for protection under the PBR Act of Canada.

2. LITERATURE REVIEW

Species Descriptions

Blue grama, the most abundant grass species of the short grass prairie, is found native on over 200 million acres across the Great Plains of North America. To date there are 39 other recorded species of *Bouteloua Lag.*, the majority occurring in North America, with some populations occurring as far south as Central and South America (Gould, 1975).

Blue grama is a native warm-season perennial grass found in diverse habitats. It is most often classified as a bunch grass (i.e. without rhizomes), but is sometimes referred to as weakly rhizominous due to its ability to slowly increase in plant size. Plants are tufted with basal leaves comprising the majority of the plant material. The basal leaves can be flat or folded, usually with narrow blades. Tillers range in height from 15-80cm, but are usually between 25-60cm (Gould, 1978). Inflorescences are cross-pollinating, with one sided spikes and spikelet numbers ranging from 1 to 60 per raceme (Gould, 1975). Blue grama is dormant in the spring, but its growth rate increases as soils warm, reaching maximum growth rate by mid-summer, and flowering from early July to early August. Blue grama grasslands provide excellent grazing material for both livestock and wildlife, as it is tolerant to close grazing and low moisture levels (Gould, 1978). However, grazing has been shown to decrease overall forage productivity (Sims et al., 1973). This species prefers course sandy loam to medium loam textured soils and neutral to slightly alkaline conditions. It also tolerates moderately saline soils (Gould, 1978).

Prairie junegrass is a dominant native cool-season perennial bunch grass found mainly in the northern boreal regions of North America and Europe. It shows preference for dry uplands with coarse to medium textured soils, but has been identified on lower mid-slopes as well as on well-drained lowlands (Hitchcock, 1950). Prairie junegrass is one of the earliest maturing grass species with above ground growth beginning around mid-April in western Canada, flowering by late May, reaching the mature seed stage by early to mid-July. Although a viable seed producer, prairie junegrass seedlings show more difficulty in surviving extreme conditions, such as summer drought and winter-kill, than other native prairie grasses. Within the first season, surviving plants may reach 7.5 – 20 centimeters in height. Some plant collections require three growing seasons to produce their first reproductive tillers. The number of reproductive tillers per plant may range from 1 on a small plant to 20 or 30 on larger plants, growing 50 to 75 centimeters in height. Prairie junegrass is considered an excellent forage grass, and usually comprises 1-3 per cent of ground cover in the more arid prairie regions. It is often found growing with blue grama, little bluestem (*Schizachyrium scoparium* [Michx.] Nash) and needle-and-thread grass (*Stipa comata* [Trin. & Rupr.]) (Weaver and Fitzpatrick, 1932).

Native Grass Establishment

Stand establishment is a significant limiting factor for stand reclamation and seed production of native grasses. Seedlings of native grass species generally display lower tolerances to extremes in environmental conditions than introduced grass species. There are many factors that play a part in establishing native grass stands, including seed viability, seed dormancy, seedling vigor, weed competition and the soil environment. The following discussion will focus on the factors affecting native grass establishment, and will concentrate on blue grama due to the larger numbers of studies conducted on this species compared to prairie junegrass.

Seed Viability

Germination is controlled by seed viability and the level of seed dormancy. Seed viability is often a function of the previous seasons' pollination rates, seed set and seed maturation (Brown, 1943). The seasonal timing of flowering can be critical to plant reproductive success, ensuring that proper conditions are present for successful fertilization, in addition to ensuring adequate time to complete seed maturation (Rathcke and Lacey, 1985). Native grasses can produce numerous flower stalks with florets of normal size, but caryopses within the florets may not develop. Sarvis (1923) reported that under average field conditions in South Dakota, only two to three blue grama seedlings emerged from several hundred seeds sown. Other studies have shown blue grama to have seed head fertility rates between 10% and 50% (Burton, 1939; Brown, 1943). Research suggests this variability in floret viability is a result of poor environmental conditions during anthesis, which have reduced pollen viability and subsequent pollination (Riegel, 1940; Wolff, 1951; Kneebone, 1957; Knowles and Baenziger, 1961; Schemske, 1977). For example, low temperatures, wet and dull weather and strong winds delay anthesis and the distribution of pollen, and thus reduce pollination and subsequent fertilization. In addition, soil moisture during flowering and seed maturation, and environmental factors that increase soil moisture, have been positively correlated to viable caryopsis production (Branson, 1941).

Previous studies have also shown significantly less seed production and poorer seed quality in remnant native grass stands compared to cultivated seed production fields (Cornelius, 1950). This reduction may be a result of the biological variability that is typical of native grass stands. Large differences in pollen shed periods and ovarian receptiveness among plants within a single population reduce successful pollination events. This results in reduced fertilization success rates and extended seed maturing periods throughout the season. Plant breeding programs have typically focused on producing cultivars with increased uniformity, shorter flowering periods and earlier seed maturing periods, to increase seed production.

Seed Dormancy

In some cases, viable seed may be inhibited from germination by dormancy controlling mechanisms (Hsu et al., 1985). Seed from most native grass species, including blue grama, big bluestem (*Andropogon gerardii* [Vitman]), switchgrass (*Panicum virgatum* L.) and Canada wildrye (*Elymus Canadensis* L.) are dormant as soon as the seeds reach maturity, and require various environmental conditions to initiate germination (Baskin and Baskin, 1998). Seed dormancy is determined by both genotype and environmental conditions during seed maturation. Methods have been developed to eliminate dormancy problems for some native seeds, including stratification and KNO_3 treatments (Coukos, 1943; Watts, 2001). Blue grama has a variable range in seed dormancy, with an average germination time between 3 and 14 days after planting (Weaver, 1930). However, stratification, and wetting and drying treatments have increased blue grama germination by 18 per cent (Watts, 2001). Aberle et al. (2000) showed similar results in eastern gamagrass (*Tripsicum dactlyodies* L.), where stratification led to the highest spring seeding germination rates. Seed treatments such as dehulling and the use of the plant hormone indoleacetic acid (IAA) have also been shown to decrease time to germination, increase adventitious root length and increase the number of tillers per plant (Roohi and Jameson, 1991).

Seedling Vigor

Previous studies have indicated that seedling vigor is often related to seed size and seed weight. Heavier seed weights are generally an indication of larger endosperms, and thus more energy reserves for growth (Johnston, 1960). Carren et al. (1987) found that heavier blue grama seeds emerge more readily from deeper depths compared to lighter seeds, confirming that seedling vigor increased with seed weight. In a study comparing planting depths of 20mm, 40mm, and 60mm in blue grama and crested wheatgrass, Hyder et al. (1971) recorded the highest emergence rates for blue grama at 20mm, while crested wheatgrass remained unaffected by planting depth. This study concluded that crested wheatgrass initiated adventitious root development at planting depth, while blue grama initiated adventitious root growth at the 2mm soil level. As a result, Hyder et al. (1971) recommended an optimal planting depth of 15mm-20mm for blue grama. Shallower plantings of 15mm or less have been successful when soil temperature and surface moisture levels are adequate for seedling emergence and adventitious root development (Love and Hanson, 1932; Murphy and Arny, 1939; Fults, 1944).

After emergence, transpiration begins and water uptake becomes an important factor in ensuring establishment. The seminal root and sub-coleoptile inter-node of blue grama have a limited capacity for water uptake and transport because of their small diameter (Newman, 1986). High water uptake capacity in seminal roots is crucial to maintain plant transpiration levels (Wilson et al., 1976) as well as the absorption of nutrients, such as phosphorous, which aids in root development (Radin and Eidenbock, 1984). However, water conducting vessels in the extremely thin subcoleoptile internode of blue grama are unable to supply adequate water to the above ground biomass (Wilson et al., 1976). As a result, the morphological development of adventitious roots is essential in establishing blue grama. Adventitious roots are longer-lived and have a greater capacity for water uptake than the seminal or primary root (Briske and Wilson, 1980). Failure to develop adventitious roots in blue grama results in low plant establishment rates (Hyder, 1974; Van Der Sluijs and Hyder, 1974; Wilson, 1981).

After approximately 2 - 4 weeks of favorable environmental conditions following emergence, seedlings are capable of developing adventitious roots from the crowns, which are located approximately 2mm below the soil surface (Weaver, 1930; Hyder et al., 1971). A minimum of 2 – 4 days of surface soil moisture is required to promote the growth and successful establishment of these roots (Hyder, 1974; Wilson and Briske, 1979). Nearly simultaneously, tiller development begins, thus increasing transpirational requirements (Weaver, 1930). If adventitious roots do not develop, blue grama seedlings usually die after 6 – 10 weeks in range sites (Wilson, 1981), but they may last 22 weeks in cultivated seed production fields (Van Der Sluijs and Hyder, 1974). Subsequent root development is dependent on soil temperatures, with warm-season grasses showing increased root extension when soil temperatures reach a minimum of 15°C (Briske and Wilson, 1980; Wilson, 1981).

Soil Residue

Soil surface conditions play an important role in regulating the soil microclimate and the germination and emergence of all native grasses. Leaving residue on the soil surface dramatically alters the microclimate from which new seedlings will emerge. Studies have shown mulches, such as straw or stubble increase blue grama emergence and establishment by conserving crucial soil moisture for adventitious root development (Fults, 1944; Glendening, 1942) and moderating temperature fluctuations. Improvements in microclimates have increased blue grama establishment rates 4 to 20-fold in arid regions (Glendening, 1942). Planting grass seed into preparatory crops in arid regions (i.e. Okalahoma), such as Sudan grass (*Sorghum vulgare* var. *sudanense* Hitchc.) or hay residues, has been shown to increase rates of seedling emergence and establishment. Residues limit sunlight from reaching soil surfaces, which regulates soil surface temperatures and minimizes evaporation (Savage, 1939; Savage and Smith, 1940). However, in more temperate regions, the increasing rate of soil warming associated with the absence of residues has been shown to improve grass seedling establishment, especially of warm-season species (Fults, 1944).

Soil Type

Previous research has indicated that soil texture (often associated with inherent fertility levels) is more important than applied fertilizer in promoting the establishment and long-term survival of certain native grass species (Power, 1980; Belanger et al., 2000). Clay loam soils typically promote the growth of mixed grass prairie species, including little blue stem, western wheatgrass (*Pascopyrum smithii* [Rydb.] A. Love; formerly *Agropyron smithii*) and Alpine knotweed (*Polygonum alpinum* All.) (Waller et al., 1975). Sandy soils commonly promote the growth of short grass species, including blue grama, prairie junegrass, and side oats grama (*Bouteloua curtipendula* [Michx.] Torr.) (Coffin and Lauenroth, 1992).

Inherent to soil texture is soil pH, which has also been shown to influence the growth of certain grass species. In a study in north-eastern Minnesota, Kentucky bluegrass stands produced 60% more biomass on an alkali pH soil, while timothy produced 36% more biomass on an acidic pH soil (Grava, 1970).

Soil Fertility

Soil fertility is an important requirement for the establishment and seed production of many native grass species (White, 1961). Poor soil fertility levels reduce native grass seedling vigor, hindering their establishment. Previous studies have shown seedling vigor and survival of native grasses increased as adventitious root development accelerated (Hyder, 1974; Van Der Sluijs and Hyder, 1974; Wilson, 1981). Research results show that phosphorus (P) is an important nutrient in promoting root development and plant establishment (Havlin et al., 1999). However, P does not occur as abundantly in northern Great Plains soils as other nutrients such as nitrogen (N). Additionally, the plant available portion of P in the soil is generally much lower than the total amount of P in the soil. Consequently, previous studies have shown that additions of P fertilizers can increase seedling survival and establishment rates of some grasses, including weeping lovegrass (*Eragrostis curvula* [Schrad.] Nees) (Cummings, 1947). Studies on little bluestem showed substantial increases in early season biomass production when 45 kg ha^{-1} of P was added (Reardon and Huss, 1965). However, P response is significantly influenced by environmental factors, including the native soil P levels. Plant responses to applied P decrease as native soil P levels increase (Olson et al., 1962), often delaying responses to applied P for up to three years (Lorenz and Rogler, 1972). Lorenz and Rogler (1972) concluded that P fertilization increased blue grama biomass production only when averaged over 8 years of application. Additionally, higher increases in blue grama biomass production were recorded when P was applied with N fertilizer. Lorenz and Roger (1973) showed that 90 kg ha^{-1} N + 20 kg ha^{-1} P on blue grama produced more above ground biomass than 180 kg ha^{-1} N alone. Other studies on Kentucky blue grass indicated similar interactions between N and P (Ebdon et al., 1999).

Soil Inoculant

In addition to adding inorganic P fertilizer, previous studies indicate certain natural occurring soil fungi can increase P availability in the soil. Field experiments have shown that *Penicillium bilaii* inoculation increased P uptake, seedling vigor, dry matter accumulation and seed yield in wheat (*Triticum aestivum* L.), canola (*Brassica napus* L), field pea (*Pisum sativum* L.) and field bean (*Phaseolus vulgaris* L.) (Kucey and Leggett, 1989; Downey and Van Kessel, 1990). However, this yield increase has been shown to be a result of an increase in root mass and subsequent soil exploration, an important component for seedling vigor, rather than an increase in plant available soil P (Gulden and Vessey, 2000; Vessey and Heisinger, 2001).

Weed Competition

Weed competition can be detrimental in the establishment of many perennial grass crops. Unlike introduced grass species, native grasses generally concentrate initial growth into root production and winter survival as opposed to above ground vegetation and seed production (Redente et al., 1989; Smoliak and Dormaar, 1985). As a result, inadequate weed suppression is the most common cause of native grass seeding failures (Duebbert et al., 1981). Reduced blue grama seedling vigor and stand densities are often a result of moderate weed competition (Riegel, 1943). Previous research has shown applications of