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

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A Thesis submitted to the Faculty of Graduate Studies
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for the Degree of

MASTER OF SCIENCE

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Master of Science

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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.

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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

selective herbicides during the growing season to control weeds can increase native grass establishment rates. Houston (1977) indicated blue grama seedling establishment was increased with an annual fall application of atrazine (2-chloro-4-ethlamino-6-isopropylamino-s-triazine) at 2 kg ha^{-1} of active ingredient. However, some studies show that weed control is not an important factor when establishing certain native grasses, such as big bluestem (Andropogon gerardii [Vitman]) and indiangrass (*Sorghastrum nutans* (L.) Nash) (Bryan and McMurphy, 1968).

Native Grass Seed Production

Native grass seed production is fundamentally limited by the establishment success of a native grass planting (Jefferson et al., 2002). However, once a stand is established, seed production is a function of the development of reproductive tillers and vegetative biomass production (Jewiss, 1972). Increased vegetative biomass provides increased quantity and subsequent availability of photosynthates for seed head meristem development. Conditions favorable for vegetative growth and synthate production support initiation of tillering and flower production. Therefore, native grass seed production is affected by many environmental conditions controlling its growth, including daylength, temperature, soil conditions (i.e. moisture availability, fertility, and texture), and the agronomic practices which may affect these conditions such as field cropping techniques. Further discussion of important native grass seed production research will

concentrate on blue grama due to the larger amount of data found for this species compared to prairie junegrass. Due to lack of information available on perennial grasses, some reference will be made to annual *Gramineae* plants, including corn and wheat.

Temperature and Daylength

There is considerable evidence indicating a relationship between the timing of tillering and reproductive fecundity of a plant (Schemske, 1977). If optimum environmental conditions are not present, axillary buds are not initiated and the plant remains in the vegetative growth stage. The development of the axillary bud into a tiller is a function of air temperature, light intensity and photoperiod (Mitchell, 1953a,b; Ryle, 1966 a,b). For example, an increase in air temperature during mid-spring growth of timothy (*Phleum pratense* L.) resulted in tiller development occurring up to 4 weeks earlier in the season (Ryle, 1966a). Earlier initiation of tiller development has been shown to increase tiller densities in tall fescue swards (Robson and Jewiss, 1968). Factors that promote conditions for earlier spring growth and tiller development may ensure adequate time for proper seed maturation and fill.

A positive correlation also exists between tillering and light intensity (Mitchell, 1953a,b). Environmental factors that increase the level of photosynthetically active radiation (PAR) reaching newly emerging leaves can increase seedling vigor. However, a negative correlation exists between tillering

and photoperiod (Mitchell, 1953a,b). Many grasses, especially C_3 species, will not initiate inflorescence until a critical daylength is reached in the spring. Exposure to photoperiods shorter than the critical daylength, or exposure to excessively high temperatures at this early stage, may cause a reversion back to the vegetative growth stage (Ryle, 1972). However, special circumstances do exist. In the case of some species, including prairie junegrass, tiller production may only occur every two or three years, regardless of photoperiods (Weaver and Fitzpatrick, 1932).

Soil Moisture

Seed production is positively correlated with the quantity of soil moisture and the timing of availability (Branson, 1941; Brown, 1943). For many species, including prairie junegrass, the moisture regime of a habitat is more important than the length of the growing season (Roberston and Ward, 1970). Blue grama is also significantly influenced by soil moisture content. Previous studies show that blue grama decreases transpiration during dry periods, resulting in slower tiller advancement and seed production (Brown, 1943). Increased seed yields were collected from remnant prairie stands of blue grama after a heavy rainfall event one month prior to harvest (Wolff, 1951). Ryle (1972) suggested that this increase in seed yield may be due to increases in seed weight, rather than increases in seed number. Conditions promoting increased photosynthetic

activity (e.g. rainfall events) will increase metabolite availability, and subsequently reduce metabolite competition between embryos.

Soil Fertility

Soil fertility is an important factor in native grass seed production. Although N is often a limiting factor in native grass production, low P levels have been shown to have comparable influences on reproductive tiller development (Caldwell et al., 1985; Cummings, 1947). Large quantities of P are found in native grass seeds (Khasawneh, 1980). Therefore, P is considered essential for native grass seed formation and maturation (Drew, 1975). Differences exist in P utilization between some C_4 and C_3 species. Previous studies have shown that corn and sorghum (C_4 species) are more efficient in utilizing soil P compared to wheat and barley (C_3 species) (Olson et al., 1962). This difference exists because root physiology of C_4 crops facilitates adequate early season P uptake, often within four weeks after planting, for season long growth (Olson et al., 1962; Barber and Olson, 1968). As a result, some C_4 crops tend to outgrow early growth responses to applied P, with little effect on final yield (Olson et al., 1962). Bates (1971) reported that P fertilization increased corn yields in 2 out of 22 field experiments in Ontario, Canada. Grava (1973) showed that an application of P fertilizer increased seed P concentrations in timothy and Kentucky blue grass (*Poa pratensis* L.) seed production fields, but did not affect seed yield. Furthermore, in-furrow and liquid-foliar applications of 20 kg ha^{-1} P did not affect

seed yield of pusa giant anjan (*Cenchrus ciliaris* L.) in India (Rai and Shankarnarayan, 1981)

However, other studies have shown that P responses can result from additional P fertilization. A pre-seeding broadcast application of 49 kg ha^{-1} P in a switchgrass stand increased seed production by 42 per cent (McKenna and Wolf, 1990). Long-term studies conducted in an undisturbed native grassland in the Mediterranean, which had low pre-fertilization P levels of 5-6ppm, showed that additions of 60 kg ha^{-1} P increased the seed size of native grasses between 1.4 to 2.5 times, with responses lasting as long as 5 years after application (Osman et al., 1999).

Previous research has also shown increases in grass seed production due to P fertilization when P was applied in combination with other nutrients. Big bluestem stands in Nebraska exhibited a 46% increase in seed production when both P and N were applied; however, this improvement was only visible when sufficient moisture conditions were present (Masters et al., 1993). P applied in combination with K significantly increased the number of seed heads in Bermuda grass (*Cynodon dactylon* [L.] Pers.). The addition of N to a P and K treatment further increased the production of seed heads in a number of grasses studied, including bahiagrass (*Paspalum notatum* [Fluegge]), carpet grass (*Axonopus affinis* [Chase]), and woollyfinger grass (*Digitaria erianth* [Steudel]) (Burton, 1943).

Method of Phosphorus Application

The method of P application is important because of its limited mobility in the soil profile. There are a range of application methods, including banded (in-furrow or side), broadcasted, and foliar applications. Banding typically results in higher effectiveness per unit of applied P by reducing the amount of P exposed to soil surfaces, and subsequent adsorption. Barber and Olson (1968) concluded that corn was more effective in absorbing season long P from in-furrow band applications compared to broadcasted applications. Rudd and Barrow (1973) found that in-furrow band applications of superphosphate in wheat in Australia were approximately twice as effective as that applied via broadcasting. Additionally, applications at seeding were three to four times more effective than fall applications. Similar differences between banding and broadcasting have been shown in corn and small grains on P-deficient soils (Prummel, 1956).

Banding an immobile nutrient such as P generally becomes less important as soil P reserves rise over time with continued application. Barber (1958) found that yield responses of corn to banded P decreased as pre-application soil P levels increased over time. Further studies comparing broadcast to band applications in corn have shown that the two methods are equal when pre-application soil-P levels are sufficient (Singh et al., 1966; Belcher and Ragland, 1972). However, broadcast applications of P have been shown to increase vegetative production in some forage species, including alfalfa, ladino clover, and orchard grass (Stanford et al., 1950).

Foliar application serves as an additional method for delivering nutrients to deficient plants. Several studies have shown that foliar-applied P is absorbed by leaves of various plants (Silberstein and Wittwer, 1951; Fisher and Walker, 1955). However, the amount absorbed at any one spraying is limited. Therefore, repeated applications may be necessary for sufficient nutrient absorption. Previous studies have shown that there are direct relationships between crop responses of foliar-applied P and the capacity of the soil to immobilize P (De Datta and Moomaw, 1965; Lancaster and Savatli, 1965). The authors of these studies concluded that although foliar applications of P increased crop yields when pre-application soil P levels were deficient, yields were not increased beyond that which would be obtained with soil applications alone.

Row Cropping

When availability of soil nutrients and water are limited, plant-to-plant competition may limit tiller growth and seed production. Modified field production practices, such as row cropping, have been shown to reduce plant-to-plant competition, and increase seed production in several native grass stands (Cornelius, 1950; Roos and Quinn, 1977). Previous studies have shown that anthesis is delayed as grass stands increase in age and plant-to-plant competition increases (Cornelius, 1950). Delayed first anthesis dates usually result in fewer reproductive tiller numbers per plant (Roos and Quinn, 1977). In a study using a well-established blue grama grass stand, chemically sprayed out

strips were applied to simulate a row-cropped stand. Subsequent forage production increased a total of 232% within the first 5 years, levelling off to a 168% increase above previous production thereafter (McGinnies, 1984). McGinnies (1971) concluded that blue grama showed a positive correlation between increasing inter-row plant space and reproductive tiller heights, number of reproductive tillers per plant, and biomass production per plant. This is supported by Mueggler (1972), who showed that herbage production of bluebunch wheatgrass (*Agropyron spicatum* [Pursh]) doubled and reproductive tiller numbers tripled when plant density was partially decreased. Herbage and reproductive tillers increased six and ten-fold respectively, when all neighbouring plants were removed. In a study conducted on little bluestem, inter-row spaces of 75 centimetres and intra-row spaces of 30 to 60cm produced higher seed yield per unit area than 37.5 and 2.5cm respectively (Cornelius, 1950).

Row cropping has also been shown to promote the influence of the 'edge effect' in seed production fields. The edge effect, as defined by McGinnies (1984), occurs when shoots located near the outer edge of the crown are larger in size and produce more reproductive tillers than those growing in the middle. Row cropping promotes higher tiller densities by creating more outer edge area within a crop stand and thus potential for higher seed production. More research is required in order to determine the relationship between cropping practices and seed production.

Residue Management

Several investigators have reported that protection of established native swards from burning, mowing or grazing results in an accumulation of litter and a subsequent reduction in stand productivity (Weaver and Fitzpatrick, 1934; Weaver and Rowland, 1952; Kelting, 1954; Kucera and Koelling, 1964). This research can also be applied to native grass seed production fields. In spring, dormant perennial grass species require moisture and heat in order to begin growth. Although residues provide increased wind protection to young seedlings, excessive protection is not desirable. Temperatures of photosynthetically active leaves in unburned stands can reach intolerable levels due to the excessive protection offered by tall standing residue (Knapp, 1984). Increased levels of residue can reduce the rate of soil warming and PAR reaching newly emerging leaves, thus shortening the available growing season, and negatively affecting seed production. Spring burning in mixed native grass stands containing big bluestem (*Andropogon gerardii* [Vitman]), little bluestem, and switchgrass increased soil temperatures by 20% due to solar warming (Rice and Parenti, 1978). Removing residues in a big bluestem prairie increased the PAR reaching emerging shoots, resulting in a 55% increase in above ground biomass production (Knapp, 1984). Balasko and Smith (1971) showed that switchgrass biomass production can increase as much as 59% when soil temperatures are increased.

Photosynthetically active radiation is also important for increasing tiller development. Previous research has shown that reduced tillering in ryegrass (*Lolium spp.*) stands is a result of reductions in PAR reaching emerging shoots (Mitchell, 1953b). In a study conducted on big bluestem, Knapp (1984) showed that residue reduced the PAR reaching emerging shoots by 58.8%, resulting in biomass reductions of 55.4%.

Previous studies have shown that increases in tiller production due to burning are more attributable to the timing of the burn event rather than soil warming, the critical period being during seed head meristem development. For example, increases in spring vigor and vegetative growth have been observed when burns were conducted in mid-spring for little bluestem and fall for prairie junegrass (Towne and Owensby, 1984). However, burn events on some cool-season species have resulted in two-year reductions of vegetative growth before an increase in plant growth is observed (Kirychuk, 2001; Cornelius, 1950).

Mowing is an alternative strategy to burning native grass stands. Mowing tends to stimulate vegetative spreading and productivity for many low growing rhizominous or stoloniferous grass species, such as buffalo grass (Savage, 1934), and for taller grasses such as little bluestem (Cornelius, 1950). However, seasonal renovation by mowing in blue grama has been shown to have both positive and negative effects on seed production. Sims et al. (1973) showed that mowing blue grama in the northeastern sandhills of Colorado in late June and early July stimulated additional tillering from reproductive tillers that were elevated above the height of cut. Mowing in late July and early August also stimulated

tillering, but the remaining growing season was insufficient to complete development of these late tillers. Earlier mowing however, tended to suppress tillering. In contrast, research in the eastern plains of Colorado showed that mowing blue grama was not useful for increasing stand density (Fults, 1944). Little bluestem showed increased seed production with prescribed mowing; however, continuous clipping throughout a season resulted in a decrease in seed production the following year (Jameson and Huss, 1971).

Plant Breeders' Rights

The History of Plant Breeders' Rights

Improved native grass cultivars are a necessary and cost-effective element in the quantitative and qualitative improvement of wildlife habitat conservation, eroded soils, and exhausted farmland reclamation (Jacobsen et al., 1994; Smith and Whalley, 2002). Breeding new cultivars of plants requires a substantial investment in labour, resources, money and time. The opportunity to obtain exclusive cultivar rights allows plant breeders a better chance of recovering their costs and accumulating the funds necessary for further investment. Exclusive cultivar rights are important to prevent others from multiplying the breeder's seed or other propagating material and selling the cultivar on a commercial scale, without recognizing the work of the breeder. Furthermore, some suggest that breeders capture only a small proportion of the benefits of Plant Breeders' Rights

(PBR) systems, compared to producers and distribution companies (Lloyd, 1995). Producers are provided with access to better and more productive cultivars, as well as reduced seed costs and improved extension services due to competition between breeders.

During World War I and II the implementation of a systematic plant breeding and effective variety protection system was first instituted, virtually mimicking industrial patent systems (The International Union for the Protection of New Varieties of Plants, 1991). However, technical difficulties were encountered when the patent systems were applied to plant varieties that reproduced sexually, therefore not creating exact replicates of themselves, and whose progeny's appearance also varied depending on environment. Between 1930 and 1960 many formats for protecting new plant varieties were attempted by a number of countries. However, rules drafted for plant variety protection in one country were not accepted in all countries, thus creating a problem when germplasm was transported between countries. In 1961, an International Diplomatic Conference in Paris formed the International Union for the Protection of New Varieties of Plants (UPOV). The agreement developed at this conference provided recognition of the rights of plant breeders on an international basis. This international agreement, or convention, was met with some opposition by European and North American countries. However, by 1990, 38 countries had accepted the new agreement. The UPOV convention originally signed in 1961, was revised in 1978 and again in 1991. The latest Canadian PBR Act was signed in 1994 and conforms to the latest version of the UPOV Convention (1991).

In Canada, the PBR Act is regulated by the Plant Breeders' Rights Office (PBRO), a division of the Canadian Food Inspection Agency (CFIA) (Canadian Food Inspection Agency, 2002). The policy underlying the PBR Act is similar to that for a patent, design, or copyright. In order for a cultivar to qualify for protection, it must adhere to four rules. The cultivar must demonstrate that it is: 1) Novel (N): having not been offered for sale or marketed prior to application, 2) Distinct (D): being measurably different from all other cultivars of the same species which are known to exist within common knowledge at the time the application is made, 3) Uniform (U): in that any variation should be predictable to the extent that it can be described by the breeder and be commercially acceptable, and 4) Stable (S): remaining true to its description over successive generations, such that its essential characteristics, as described in the original description for which rights were granted, are exhibited in further generations of seed or other propagating material (Canadian Food Inspection Agency, 2002).

In many countries the introduction of new cultivars and their availability declines after PBR is introduced (Hankin, 1999). Before PBR, newly developed cultivars were quickly picked up by 'competitors' and easily distributed to areas out of reach to the developing company's marketing area. Civil Society Organizations (CSOs) argue that the fundamental issues behind PBR (i.e. Intellectual Property (IP) on plant cultivars) are detrimental to the sustainability of the agricultural research and farming communities. The Action Group on Erosion, Technology and Concentration (ETC), (formally the Rural Advancement Foundation International - RAFI), an international non-governmental organization

(NGO) headquartered in Winnipeg, Manitoba, Canada, and Australia's Heritage Seed Curators' Association (HSCA), a non-profit incorporated organization, are two such organizations. Organizations such as these monitor the validity of PBR applications, often challenging PBR applications from foreign researchers on indigenous plant material. CSO's have emphasized concerns regarding the potential difficulty of proving distinctness between cultivars when intra-cultivar diversity is encouraged. CSO's have also debated the validity of allowing different testing procedures in other UPOV member countries. There are two main systems within UPOV-member countries for testing the identity of new cultivars. Under Central Testing, the breeder is required to complete a simple technical questionnaire covering only qualitative characteristics. The cultivar is then tested for Distinctness, Uniformity and Stability (DUS) by an official authority in a centralized location against all other known reference cultivars. Under Breeder Testing, the breeder makes a full description of the new cultivar based on their own DUS trials, comparing it with the most similar cultivar. Once the description is accepted by the PBRO, a federal observer is sent to verify the application and taxonomic description. Breeder Testing is much less expensive and therefore more widely used within the UPOV-member states. Originally a requirement for all UPOV members, Central Testing is now only used in South Africa, Chile and throughout most of Europe.

Debates initiated by CSO's have encouraged more stringent testing procedures within, and in addition to, the existing PBR requirements (Action Group on Erosion, Technology and Concentration, 2001; Australia's Heritage

Seed Curators' Association, 2001). Recently, a re-introduction of centralized testing has occurred in the USA, where centralized testing arrangements are organized informally by the breeders themselves for the major grass groups. Each breeder organizes and manages a test for one species in particular (Loch, 1998).

Additionally, recent advances in molecular genetics have provided useful tools for determining genetic distinctness. The development of Random Amplified Polymorphic DNA (RAPD) markers has provided a powerful tool for the investigation of genetic variation. The RAPD procedure works with genomic markers and requires relatively small amounts of DNA. As a result, it is simpler, less costly, and less labour intensive than other DNA marker methodologies. RAPD's can readily identify germplasm sources and be used to construct phylogenies of commercial cultivars, thereby differentiating between cultivars of a plant species. The RAPD markers also closely reflect the breeding histories of a wide range of germplasms, including commercial cultivars (Huff et al., 1993; Huff, 1997). Although not a replacement of conventional testing, this technology will provide a useful supplement to morphological and agronomic data in the identification of varieties.

Variability of Native Grass Populations

Most native grass species are cross-pollinated and, as a result, inherently display a wide range of phenotypic and genotypic diversity within given traits

(Gould, 1975). The variability of phenotypic and genotypic traits within and among populations is maintained via cross-pollination and genetic recombination (Wipff, 1996), and through a range of within-species ploidy levels in certain species (Snyder & Harlan, 1953; Harlan, 1958; Vogel and Pedersen, 1993; Smith and Whalley, 2002). Native grass species have developed specific levels of population variability through evolutionary processes (e.g. mutation, migration, random drift and natural selection) as they are influenced by climatic factors such as precipitation, temperature and daylength (Cooper, 1963), and soil related factors such as soil texture, soil fertility and soil depth (Dix and Butler, 1960). The development of population variability is important when considering plant biology for the purpose of long-term, low input reclamation and conservation plantings.

It is important to distinguish between variability due to genotypic diversity and that which is a phenotypic response to the environment. If variability in native grass populations is due to environment, there should be little difference between populations when grown under the same environmental conditions (Riegel, 1940). However, if variability is genetically controlled, populations should display distinct ranges in characteristics, often attributable to the response to the environment of their native habitat, including daylength, temperature, precipitation, and soil texture (Cooper, 1963; Huff et al., 1998).

Population variability allows native grass populations to adapt to adverse conditions, including environmental extremes and disease and insect pressures. Population variability also promotes the development of distinct geographical adaptations within a single species. Understanding and describing population

variability, and the distinct environmental preferences that develop, becomes especially important when differentiating between two heterogeneous populations. Previous research has suggested that population variability in native grasses varies independently of geographical range. For example, attempts to classify plants within the genus bluestem (*Schizachyrium* [*Poaceae*: *Andropogoneae*]) on regional scales have proven inconclusive (Wipff, 1996). Knapp and Rice (1996) have shown blue wildrye (*Elymus glaucus* [Buckley]) populations display no relationship between geographical distance and the degree of population divergence, illustrating that morphological variation attributed to both within and between populations was nearly equal. Furthermore, previous studies have shown the majority of variability in cross pollinating grasses is attributed to within-population variability (85.2%), with only minor amounts being attributed to between-population variability (14.8%) (Hamrick and Godt, 1989; Kubik et al. 2001).

In addition to phenological studies, previous genetic composition studies have also indicated that habitat is more influential in determining population variability in grasses, rather than geographical distance. Slender wild oat (*Avena barbata* [Pott.] ex Link) populations have been shown to display consistencies in allele distribution related to environment (Hamrick and Holden, 1979). *A priori* habitat ratings based on environmental conditions accurately predicted allele frequencies in each population. Populations occurring on infertile, xeric sites were usually monomorphic for a specific set of alleles (the xeric combination) while plants growing on fertile, mesic sites were typically monomorphic for a

specific complimentary combination of alleles (the mesic combination) (Hamrick and Allard, 1975).

Genetic distinctness between populations of little bluestem within a single region has been shown to be equal to or greater than distinctness between regions, with the most influential factor being soil fertility levels (Huff et al., 1998). Genetic analyses using RAPD markers have demonstrated buffalo grass (*Buchloe dactyloides* [Nutt.] Engelm.) has considerable genetic distinctness within populations, in addition to between populations (Huff et al., 1993). Phan and Smith (2000) found that RAPD markers revealed significant phenotypic and genotypic distinctness between native grass populations of blue grama and little bluestem within a region and their respective derived cultivars, including agronomic traits such as seed and biomass production, culm heights and days to anthesis.

Variability is also important when considering the development of population-specific environmental requirements affecting plant growth, such as temperature regimes, precipitation patterns, and daylength (Cooper, 1963). Previous studies have indicated that distinct daylength requirements between plant populations within a single species are important for the development of adaptation zones in native grass populations. For example, northern European populations of perennial rye grass (*Lolium perenne* L.) have evolved with their primary period of photosynthetic activity and carbon assimilation during the summer months, when moisture is not limiting and temperatures are favorable. In contrast, the main period of photosynthetic activity of Mediterranean populations

of perennial rye grass has evolved during the winter months, avoiding the hot and dry conditions present during the summer months (Cooper, 1963). As a result, northern European populations have evolved to initiate inflorescence under long daylength regimes, and Mediterranean collections under short daylength regimes. Similarly, certain native species are also affected when grown out of their adaptive regions. Blue grama, prairie junegrass, and little bluestem tend to flower earlier, grow less vigorously, and produce less forage when moved south of their adaptive range (Savage, 1939; Duebbert et al., 1981; Pahl and Darroch, 1997).

Ecovar™ Development

Limited seed supplies of native grass cultivars have promoted the use of introduced grass cultivars for the purpose of reclamation and conservation plantings. Most introduced grass cultivars are selectively bred to achieve uniform plant growth, increased biomass production, and enhanced seed production compared to remnant native grass stands. However, recent studies have shown that cultivars of some species, including tall fescue, may gain phenotypic diversity after planting as they adapt to edaphic and climatic conditions, sometimes surpassing the levels of phenotypic diversity observed in their establishment period (Vaylay and van Santen, 2002; Rouf Mian et al. 2002). In fact, significant levels of phenotypic diversity have been observed in many newly developed introduced grass cultivars, compared to older cultivars, including timothy, tall

fescue and smooth bromegrass (Casler, 2001; Vaylay and van Santen, 2002; Massa et al., 2001).

The establishment of introduced grass cultivars for reclamation purposes has been successful; however, studies during the last 30 years have shown that introduced grass cultivars are often not persistent, and require regular re-seeding and maintenance (Duebbert et al., 1981). Furthermore, although some present cultivar selection techniques include increased levels of genetic diversity for improving stand longevity and long-term productivity (Acharya and Huang, 2000; Watson Jr et al., 1995; Sellmann, et al., 1997), their relative uniformity in comparison to remnant prairies and some native grass cultivars has been linked to reductions in prairie habitat and biodiversity (Wilson and Belcher, 1989). As a result, reclamation and conservation plantings since the late 1970's have promoted the development and use of native grass cultivars rather than introduced grass cultivars, attempting to use native species to provide the necessary diversity requirements for reducing losses in plant and wildlife biodiversity (Jacobsen et al., 1994).

Native grass species are often better adapted to North American climates than European and Eurasian grass species; however, the successful establishment of many native grass cultivar plantings has been limited primarily by poor seedling vigor (Brown, 1943; Kneebone, 1957; Rathcke and Lacey, 1985). Although most native grass cultivars have relatively more genetic variability than introduced grass cultivars, problems in stand establishment have also been linked to the narrow range in environmental adaptability found in many

native grass cultivars (Jacobsen et al., 1994). Native grass cultivar development is typically based on selecting the best performing plants (e.g. for seed production or seedling vigor) from a nursery population, disregarding the phenotype's regional adaptations (Phan and Smith, 1997a&b; Phan and Smith, 1998). Some native grass cultivars have been developed from the seeds of a single plant (Hanson, 1972). Although the resulting native grass cultivars have improved seedling vigor and seed production, they are also uniform, have little biological variability, and are restricted in their environmental adaptations. As a result, the use of seed produced from some native grass cultivars is geographically limited to within 450 kilometers north and south of its production, and 300 meters in altitude (Cooper, 1957; Allard, 1970; Jacobsen, 1984; Jacobsen et al., 1994). Although, in the absence of altitudinal change, some native grass cultivars have been grown successfully in areas 1000km from their origins (Thornburg, 1982). If planted outside of their adaptive region, native grass stands often show reduced persistence, and require additional inputs within a few years. For example, native grass cultivars of blue grama, little bluestem, big bluestem, prairie sandreed (*Calamovilfa longifolia* [Hook] Scribn.), sand dropseed (*Sporobolus cryptandrus* [Torr.] A. Gray), and indiangrass which were developed in Kansas and Colorado, showed establishment rates between 0 and 20% when planted in Saskatchewan, Canada (Kilcher and Looman, 1983). Kilcher and Looman (1983) concluded that the locally adapted plant material included in the study; including needle-and-thread grass and rough fescue (*Festuca campestris* [Rydb.] and *F. hallii* [Vasey]

Piper) established more successfully and maintained more vigorous growth in comparison to the southern adapted cultivars for the duration of the experiment.

In contrast to many cultivar selection methods, the ecovar™ selection method is based on selecting plants for one or more improved traits with equal representation from all collections included in the nursery population (Phan, 1997a,b). Ecological varieties were first suggested by Erling T. Jacobsen of USDA-NRCS and are being developed to combine genetic diversity with minor improvements in plant characteristics (e.g. seed production and seedling vigor). An ecovar™ represents an intermediate step between a local native plant collection and a cultivar. The selection technique ensures a seedlot is comprised of germplasm from a range of environments, each with unique phenotypic and genotypic adaptations. As a result, the selection technique inherent to ecovar™ development may more consistently provide widely adapted seed stocks and increased potential for biodiversity over the narrowly selected native grass cultivars.

Currently, it has not been determined if ecovars™ will qualify for protection under the PBR Act due to their biological variability and the potential difficulty in describing their uniformity. Additionally, if adequate population uniformity is achieved for taxonomic description, concerns exist around the ability of native grass breeding populations and germplasm to maintain their increased adaptability and longevity over cultivars (Action Group on Erosion, Technology and Concentration, 2001; Australia's Heritage Seed Curators' Association, 2001). However, numerous studies have shown that distinctness is possible between

two highly variable populations (Casler, 2001; Massa et al., 2001; Vaylay and van Santen, 2002; Rouf Mian et al. 2002). Research conducted by Phan and Smith (2000) showed derived populations of both blue grama and little bluestem ecovars™ to be genetically distinct from native populations. Although distinct from native populations, ecovar™ populations of both species showed similar levels of within-population variability compared to native stands. Studies conducted on perennial ryegrass also showed that although distinct, derived cultivars retained comparable ranges in biological variability to original populations (Huff, 1997). This suggests that the maintenance of within-population genetic variability may be compatible with the presence of between-population distinctness in spite of annual exchanges in gene pools.

The effects of soil texture, seeding rate, phosphorus fertilizer and *Penicillium bilaii* on blue grama [*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths] and prairie junegrass [*Koeleria macrantha* (Ledeb.) J.A. Schultes] establishment.

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ABSTRACT

The interest in native grasses continues to grow across North America for conservation, reclamation, Conservation Reserve Programs, right-of-ways, and wildlife habitat, but low seed availability and poor seedling vigor currently limits stand establishment.

The objective of this study was to assess the effect of seeding rate, phosphorous fertilizer, a *Penicillium bilaii* seed treatment and soil type on the establishment of blue grama (*Bouteloua gracilis* [H.B.K.] Lag. ex. Steud.) and prairie junegrass (*Koeleria macrantha* [Ledeb.] J.A. Schultes) ecovars™.

Tests were conducted in a controlled environment and measurements included seedling establishment, plant height and leaf stage. P treatments were applied in-furrow and observed for 6 weeks. A liquid N application was made six weeks after planting; followed by a final measurement of plant height, leaf stage and biomass production eight weeks after planting.

We observed that a seeding rate of 200 seeds meter^{-1} row increased row densities compared to 100 seeds meter^{-1} row, but decreased the percentages of seedling establishment (measured as the number of surviving seedlings per seeds planted) for both blue grama and prairie junegrass. There was no increase in seedling establishment in response to P fertilizer application, *Penicillium bilaii* inoculation or foliar-N application. However, both species responded similarly to changes in soil type, with the sandy loam providing the highest seedling

establishment and largest plants compared to the sandy clay loam and loamy sand soils.

These results suggest that establishment of blue grama and prairie junegrass is limited more by soil type than from P and N fertilization.

INTRODUCTION

Native grasses, defined as those grass species indigenous to North America prior to European settlement, were the predominant vegetation in the North American Great Plains and formed the tall grass, mixed grass and short grass prairie regions. Blue grama, and prairie junegrass are two predominant native short grass prairie species found traditionally throughout the Great Plains (Gould, 1975).

The drought and soil erosion problems of the 1930's stimulated interest in reclaiming the highly erodable lands on the Northern Great Plains of North America. Most revegetation efforts have focused on the use of introduced European and Eurasian grass species like tall fescue (*Festuca arundinacea* Schreb.), smooth brome grass (*Bromus inermis* Leyss.) and crested wheatgrass (*Agropyron cristatum* L.) because of their ease in establishment and plentiful seed production (Jacobsen et al., 1994). Studies over the last 30 years have shown that many introduced grass stands require regular replanting and maintenance, thereby increasing long-term costs (Duebbert et al., 1981). Previous studies have concluded that once established, native grasses are more tolerant to environmental extremes and require fewer long-term inputs (Jacobsen et al., 1994). However, successful establishment of many native grass plantings has been limited due to low seed viability, high levels of seed dormancy, and poor seedling vigor, which has compromised their short-term competitiveness (Brown,

1943; Kneebone, 1957; Rathcke and Lacey, 1985; Hyder et al., 1971; Smith and Whalley, 2002).

Although seed treatments, including stratification and KNO_3 have been proven to be successful methods for reducing dormancy and increasing germination rates in some native grasses, including little bluestem, green needlegrass and prairie sandreed, these treatments have been inconsistent in reducing dormancy in blue grama (Coukos, 1943; Watts, 2001).

Previous studies have shown that P is an important nutrient in promoting root development and plant establishment (Havlin et al., 1999). Early season additions of inorganic P have increased the seedling vigor, establishment success, biomass production, and seed yield of many native grasses, including blue grama (Cummings, 1947; Reardon and Juss, 1965; Lorenz and Rogler, 1972). Additionally, seed inoculations of *Penicillium bacilli*, a soil borne fungus which has been shown to increase P uptake, have increased seedling vigor, biomass production and seed yield in a range of crops including wheat, canola and field pea (Kucey and Leggett, 1989; Downey and Van Kessel, 1990). However, previous research has shown that soil type is more influential in promoting native grass establishment than soil fertility (Power, 1980; Belanger, 2000). Clay loam soils typically promote the growth of mixed grass prairie species, including little bluestem (Waller et al., 1975), while sandy soils commonly promote the growth of short grass species, including blue grama and prairie junegrass (Coffin and Lauenroth, 1992).

The objectives of this study were to assess the effects of seeding rate, phosphorus availability, and soil texture on the establishment of blue grama and prairie junegrass ecovars™.

MATERIALS AND METHODS

Ecovar™ Development:

In late July, early August of 1992, 495 live blue grama plants were collected from 11 locations in southern Manitoba. The collection sites were 30 km apart. The collections were transplanted into 9 blocks at the University of Manitoba research station in Winnipeg, Manitoba in early August. The blue grama ecovar™ was selected from this nursery. The blue grama ecovar™ selection method involved selecting the highest seed yielding plants from each collection site within each block for a total of 99 plants, resulting in a 20% selection intensity. The selections were planted into isolated spaced-plant crossing blocks in 1996 for the prebreeder seed production. The prebreeder seed harvested from these plots was replanted to produce breeder seed, which was used for this experiment. The ecovar™ development procedure is described in detail by Phan and Smith (2000).

In 1993, 1000 live prairie junegrass plants were collected from 20 locations (50 plants per location) in southern Manitoba. The difference among collection sites was primarily based on the geographical distance between sites,

maintaining at least a 30km distance between sites. The secondary objective was to include collection sites with different soil types to ensure that plants from a range of soil types were included in the collection. The collections were transplanted into a 5-block spaced-plant nursery at the University of Manitoba research station in Winnipeg, Manitoba. The prairie junegrass ecovar™ was selected from this nursery. The prairie junegrass ecovar™ selection method involved selecting the highest seed yielding plant from each collection site and block for a total of 100 plants, resulting in a 10% selection intensity. Selected plants were planted into isolated crossing blocks for the prebreeder seed production. The seed harvested from these plots was replanted to produce breeder seed, which was used for this experiment.

Experimental Design

Seedling establishment trials for the blue grama and prairie junegrass ecovars™ were planted under controlled environmental conditions in a growth chamber (Conviron, Winnipeg)[Ψ] at the University of Manitoba in Winnipeg, Manitoba, Canada. Temperatures in the growth room were set at 22°C/day and 15°C/night, with a 16/8-hour day/night regime. Seeds were planted in wooden screen-bottom boxes measuring 165cm x 30cm x 13cm, with a soil depth of 8.75 – 10cm. Two soil types were collected from the University of Manitoba Carman Research Station. The first soil type was a sandy-clay loam (SCL), comprised of

[Ψ] Controlled Environments Limited 590 Berry Street, Winnipeg, Manitoba, Canada, R3H 0R9

51% sand, 26% silt and 23% clay measured by weight, with a pH of 5.1, an organic matter content of 4% and a soil P concentration of 33ppm. The second soil type was a loamy sand (LS) comprised of 88% sand, 7% silt, and 5% clay, with a pH of 7.9, an organic matter content of 2% and a soil P concentration of 21ppm. Both soils were screened to remove large particles of organic matter and soil aggregates. Mixing approximately equal portions of the SCL and LS soils created a third soil type, a sandy loam (SL). This soil type was comprised of 68% sand, 17% silt, and 15% clay, with a pH of 5.4, an organic matter content of 5% and a soil P concentration of 32ppm. The experiment was conducted in a series of 3 trials, one each in each soil type. The initial design of this experiment included a series of 3 trials using the same soil type. However, there was not sufficient soil for all three trials, and thus more soil was obtained from the University of Manitoba Carman Research Station for the second and third trials. Because the second soil type was of a different texture than the first soil, approximately equal proportions of the first and second soil types were mixed to create the third soil type.

The treatments were two seeding rates: S_1) 100 seeds m^{-1} of row and S_2) 200 seeds m^{-1} of row, and four P treatments, three of which were in-furrow P fertilizer applications and one a fungal inoculant: F_1) 0 kg P ha^{-1}, F_2) 33 kg P ha^{-1} and F_3) 66 kg P ha^{-1} applied as 0-46-0, and F_4) an inoculant seed coating of Jumpstart™♣, applied alone at 7.86 x 10^5 cfu *Penicillium bilaii* per gram of blue

♣ Philiom Bios, 318-111 Research Dr., Innovation Place, Saskatoon, SK., Canada. S7N 3R2.

grama seed, and 1.1 x 10^6 cfu *Penicillium bilaii* per gram of prairie junegrass seed. The eight treatments for seeding rate x P were established across 12 boxes for each soil type, with 4 replications of each treatment arranged in a Completely Randomized Design. Each treatment was applied in groups of three rows, each 12cm long, and all measurements were taken from the middle row to account for fertilizer movement between adjacent treatments. Furrows were made with a 3mm wide wooden ruler pushed into the soil producing a uniform planting depth of 2cm. Furrows were filled and packed with a 2cm wide ruler using uniform hand pressure. Jumpstart™ inoculant was suspended in a water solution and misted onto the seed with a spray bottle, while the seed was continuously agitated by hand. Visual confirmation of the uniform application of the inoculant on the seed was ensured by the use of a green dye included in the inoculant package. Inoculant was allowed to air-dry before planting the seed. Boxes were watered every 2 days from above until water ran through the screen on the bottom of the box. Weeds were removed by hand. After 6 weeks of growth each box was sectioned off length-wise, dividing each row in half, and a liquid foliar application of nitrogen (N) was applied to one half of the row in a split-plot design. Urea was dissolved in water and applied at 22 kg N ha^{-1} with a hand spray bottle.

Measurements

Using the center row of each treatment, the following measurements were collected every 3 days during the six-week growth period: number of seedlings, average plant height, and leaf stage. The total number of seedlings per row, including newly emerged and those surviving from the previous count, was used to represent the row density of each treatment. Seedling establishment was calculated by expressing the row density as a percent of the total number of seeds planted. Average plant height was calculated as the average height of three randomly selected plants in the center row of each treatment. Average leaf stage was determined by calculating the average leaf stage of three randomly selected plants in the center row of each treatment. Leaf stage was advanced at the first appearance of a new leaf. At the first appearance of tiller development, the leaf stage was advanced to 10. An additional value of 10 was added to the leaf stage count at the initial appearance of each additional tiller thereafter. Two weeks after the foliar-N was applied, final height and leaf stage measurements were taken. Above ground biomass of each center row was hand harvested and dried at 80°C for 24 hours and weighed to determine biomass production per treatment.

Statistical Analysis

Data were subjected to an Analysis Of Variance (ANOVA) using the General Linear Model (GLM) procedure of SAS version 8.02 (SAS Institute, 1988). Height and leaf stage raw data were transformed using a logarithmic function because of the exponential increases in character magnitudes and the associated increase in variability. The transformation served to equalize variability across measurement days. Transformations were calculated as follows: $Y=\log_{10}(X+1)$ where X is the raw character measurement (Ott, 1988). Transformed Least Squared Means were back-transformed for each treatment for use in the tables. Fisher's protected Least Significant Difference (LSD) test was used to compare means at the 0.10 probability level, which compared means when the ANOVA F-test was significant. As a repeated measure design, all observation days (1-42) were included in the analysis, averaging each of the characters measured over the 42-day experiment. The soils were tested and determined to have marginal to optimal levels of P before the additional P treatments were applied (Norwest Labs, 2000). Because of the adequate pre-fertilization P levels, a significant response to the P treatment was considered at the 0.10 probability level, rather than the most often used 0.05 probability level. To ease illustration and explanation of data, other treatments were also deemed significant at the 0.10 probability level.

Statistical model used in ANOVA analysis for testing effects of soil type, seeding rate and P treatments on establishment of blue grama and prairie junegrass in a controlled environment.

Source	DF	Fixed Effect	Random Effect
Soil type	2	X	
Seedrate	1	X	
Fertility	3	X	
Soil type * Seedrate	2	X	
Soil type * Fertility	6	X	
Seedrate * Fertility	3	X	
Soil type * Seedrate * Fertility	6	X	
Rep (Soil type * Seedrate * Fertility)	24		X

RESULTS

Neither two-way nor three-way interactions between soil type, seeding rate, and P treatments were significant for row density, plant height, or leaf stage accumulation of either blue grama or prairie junegrass in the initial six weeks of the experiment. Soil type had a significant effect on blue grama leaf stage accumulation after eight weeks of growth; however, foliar N, seeding rate and P treatments had no affect on plant height, leaf stage accumulation or biomass production for blue grama and prairie junegrass (data not shown). Treatment effects and interactions of the final two weeks of the experiment will not be discussed because soil type effects in the final two weeks were not different than those found in the initial six weeks of observations, and only two treatment interactions were observed in blue grama, (i.e. foliar N x seeding rate and foliar N x soil type).

Row Density and Seedling Establishment

Row density for both species was affected by seeding rate, with density increasing by 40% for blue grama, and 45% for prairie junegrass in the 2x seeding rate treatment (Table 3.1). Seedling establishment of blue grama and prairie junegrass was also affected by seeding rate (Table 3.1). Again, both species responded to the 2x seeding rate similarly, with prairie junegrass seedling establishment decreasing by 16% and blue grama by 11%. When averaged over the two seeding rates, prairie junegrass had establishment rates and row densities 66% and 67% higher than blue grama.

Neither row density nor seedling establishment were influenced by the phosphorus fertilizer or fungal inoculant treatments for blue grama or prairie junegrass (Table 3.2).

Seedling establishment for blue grama and prairie junegrass was affected by soil type (Table 3.3). Seedling establishment of blue grama was highest in the SL soil type, with a maximum seedling establishment of 50%, with the lowest establishment of 15% observed in the LS soil type. Prairie junegrass also had the highest establishment in the SL soil type (83%) (Table 3.3).

Plant Height

Plant height of blue grama and prairie junegrass was not affected by seeding rate or P fertilizer and fungal inoculant treatments (Table 3.1 & 3.2).

However, plant height was significantly affected by soil type (Table 3.3). The SL soil provided optimum conditions for blue grama growth, producing the tallest plants (74mm) in the study. The shortest blue grama plants were observed in the SCL soil, with the LS soil producing plants of intermediate height. The SL and LS produced the tallest plants for prairie junegrass. Similar to blue grama, the shortest prairie junegrass plants were observed in the SCL soil.

Leaf stage

Physiological development, as measured by leaf stage, was not affected by seeding rate for either blue grama or prairie junegrass (Table 3.1). Physiological development was also not affected by the phosphorus treatment (Table 3.2). However, physiological development of blue grama and prairie junegrass was affected by soil type (Table 3.3), with both species responding similarly. The SL soil type provided optimum conditions for the development of both species, and the SCL soil type providing the least favorable conditions.

Table 3.1. Seedling establishment, plant height, and leaf stage of blue grama and prairie junegrass plants in two seeding rates (100 seeds m^{-1} and 200 seed m^{-1}) averaged over a 6-week growth period in a controlled environment.

	Blue grama				Prairie junegrass			
	Seedling Establishment[†]	Row Density[‡]	Plant Height	Leaf stage	Seedling Establishment	Row Density	Plant Height	Leaf stage
	--(%)--	-(seedlings row^{-1})-	--(mm)--		--(%)--	-(seedlings row^{-1})-	--(mm)--	
Seeding rate	**		ns[§]	ns	**		ns	ns
100 seeds m^{-1}	36.3*a*[ʃ]	36.3	57.9	4.6	59.5*a*	59.5	35.9	4.6
200 seeds m^{-1}	25.5*b*	51.0	53.9	4.7	43.2*b*	86.4	35.6	4.4
LSD	8.9		6.5	0.7	13.8		2.3	0.6

[†] Live seedlings (including newly emerged and those surviving from previous count) measured as a percent of seeds planted.
[‡] Total number of seedlings per row, including newly emerged and those surviving from previous count, calculated from the seedling establishment data.
[ʃ] Means within columns followed by the same letter are not significantly different.
* Significant at the 0.10 probability level.
** Significant at the 0.05 probability level.
*** Significant at the 0.001 probability level.
[§] ns = not significant.

Table 3.2. Seedling establishment, plant height, and leaf stage of blue grama and prairie junegrass plants in four phosphorus availability treatments (0, 33, 66 kg P ha^{-1} and a *Penicillium bilaii* inoculation) averaged over a 6-week growth period in a controlled environment.

	Blue grama			Prairie junegrass		
	Seedling Establishment[‡]	Plant Height	Leaf stage	Seedling Establishment	Plant Height	Leaf stage
	--(%)--	--(mm)--		--(%)--	--(mm)--	
P treatments[†]	ns[§]	ns	ns	ns	ns	ns
0 kg ha^{-1} P	27.7[f]	50.6	3.9	61.2	35.9	4.5
33 kg ha^{-1} P	29.9	57.8	4.9	47.9	36.4	4.9
66 kg ha^{-1} P	33.4	58.9	5.3	49.6	34.4	3.9
Penicillium bilaii	32.9	56.9	4.4	45.7	36.4	4.8
LSD	9.3	9.2	1.6	16.9	3.2	1.3

[†] Treatments: P fertilizer applied in-furrow as 0-46-0. *Penicillium bilaii* (Jumpstart™) applied alone as a seed coating.
[‡] Live seedlings (including newly emerged and those surviving from previous count) measured as a percent of seeds planted.
[§] ns = not significant.
[f] Means within columns followed by the same letter are not significantly different.

Table 3.3. Seedling establishment, plant height, and leaf stage of blue grama and prairie junegrass plants in three soil types averaged over a 6 week growth period in a controlled environment.

	Blue grama			Prairie junegrass		
	Seedling Establishment †	Plant Height	Leaf stage	Seedling Establishment	Plant Height	Leaf stage
	--(%)--	--(mm)--		--(%)--	--(mm)--	
Soil Type	*	***	**	***	***	*
Sandy clay loam (SCL)	24.6*b*‡	34.7*c*	1.9*c*	22.6*b*	24.3*b*	1.6*c*
Sandy loam (SL)	50.1*a*	74.4*a*	5.4*a*	83.5*a*	42.7*a*	5.3*a*
Loamy sand (LS)	15.6*c*	51.5*b*	5.3*b*	32.2*b*	34.8*a*	5.1*b*
LSD	6.8	14.7	0.1	24.4	7.9	0.2

† Live seedlings (including newly emerged and those surviving from previous count) measured as a percent of seeds planted.
‡ Means within columns followed by the same letters are not significantly different.
* Significant at the 0.10 probability level.
** Significant at the 0.05 probability level.
*** Significant at the 0.001 probability level.

DISCUSSION

Previous studies have shown that native grass seed characteristics including low seed viability, high seed dormancy, and poor seedling vigor reduce blue grama and prairie junegrass plant densities, hindering their establishment (Brown, 1943; Kneebone, 1957; Rathcke and Lacey, 1985). Higher seeding rates are often used to ensure adequate plant densities. Under controlled environmental conditions, doubling seeding rates increased row densities for blue grama and prairie junegrass. However, the doubled seeding rate reduced seedling establishment for both species. The difference in final row densities between soil types suggests that different optimum row densities exist for each species x soil type combination. Differences in seedling establishment between soil types were much more apparent for blue grama. Blue grama establishment may have been reduced because of less than optimal growth chamber temperatures (15-22°C) for this warm season species. Previous research has shown differences in plant growth and establishment between C_4 and C_3 species when grown under the same controlled environment. Although Fulbright et al (1985) showed that blue grama and green needle grass (C_3), maintained equal relative growth rates at temperatures between 15-20°C, blue grama relative growth rates increased to 35% above that of green needle grass when growth chamber temperatures were increased to 20-25°C.

Blue grama and prairie junegrass exhibited their highest establishment counts in the SL soil type. Lowest seedling establishment was observed in the

LS soil for blue grama, and in the SCL and LS soils for prairie junegrass. This range in establishment between soil types can be attributed to the qualities of each soil type. Previous studies have concluded that certain soil characteristics promote the establishment and growth of different species (Waller et. al., 1975; Coffin et. al., 1992). Blue grama and prairie junegrass are typically found growing on dry uplands containing course to medium textured soils with good drainage (Gould, 1975). The SL soil type may resemble the soil found in each species' native habitat more closely than the SCL and LS soil types.

SL soil may also provide better germination and emergence conditions compared to SCL and LS soil types. Coarse textured soils generally do not retain as much moisture as fine textured soils, resulting in a rapid dry-down (Eilers et al., 1977). This was apparent by visual observation in this study, as the LS soil was drier before each consecutive watering compared to the SCL and SL soil types. The rapid dry-down in the LS may have caused the reduction in blue grama row density compared to the SL soil type (34%). A dramatic reduction of row density in the LS did not occur for prairie junegrass, and suggests that blue grama was more affected by rapid dry-down than prairie junegrass. Similar differences in plant responses to dry soil conditions have been recorded for little bluestem, sand bluestem (*Andropogon hallii* Hack.) and prairie sandreed (*Calamovilfa longifolia* [Hook] Scribn.) (Awada et al., 2001). The between-species difference in soil dry-down response, such as was observed between blue grama and prairie junegrass, may be due to the different adventitious root development zones for each species. Previous students have shown blue grama

initiates growth of adventitious roots within 2mm of the soil surface, whereas most other species initiation adventitious root growth at the depth of seeding (Everson and Bement, 1971). The development of adventitious roots is essential for the survival of blue grama, as its seminal roots and sub-coleoptile internode have limited capacity for water uptake and transport because of their small diameter (Newman, 1986). Without sufficient moisture at the soil surface, adventitious roots do not develop (Hyder, 1974; Van Der Sluijs and Hyder, 1974; Wilson, 1981). As a result, soils that promote quicker water infiltration would move water out of reach from young blue grama plants, and in particular the developing adventitious roots, thereby reducing blue grama establishment success. This is supported by numerous studies showing that shallow plantings (<15mm) are only successful when sufficient moisture is available at the soil surface (Love and Hanson, 1932; Murphy and Arny, 1939; Fults, 1944). The findings of this study agree with those of other studies, showing that blue grama seedling vigor is relatively more sensitive to low moisture conditions in the early season compared to prairie junegrass.

As SCL soils generally retain more moisture than SL soils (Eilers et al., 1977), the seedling establishment of blue grama and prairie junegrass in the SCL soil type was likely not limited by moisture availability. However, visual observation in this study showed that the SCL soil had an increased incidence of soil surface crusting after each consecutive watering. Soil surface crusting in the SCL may have delayed the seedling establishment, and ultimately decreased the establishment success of blue grama and prairie junegrass. A delay in

establishment is also likely responsible for the slower physiological development observed in both species in the SCL soil type. Further study of the influence of soil type on establishment is needed before agronomic recommendations regarding soil type suitability are made.

P is an important nutrient in promoting root development and plant establishment (Havlin et al., 1999). P fertilizer has been used to increase early stand establishment and seedling vigor of introduced and native grasses (Hyder, 1974; Wilson, 1981; Grant et al., 2000). Studies on a number of native (i.e. little bluestem) and introduced (i.e. weeping lovegrass [*Eragrostis curvula* (Schrad.) Nees]) grasses showed substantial increases in seedling survival and growth when P fertilizer was added (Cummings, 1947; Reardon and Huss, 1965). However, previous studies have also shown neutral responses to P treatments for many annual and perennial crops, attributed to biological variability within the plant population (Bates, 1971; Grava, 1973). This study also showed no responses to P treatments; however, it is not clear if biological variability played a role in the results. It is more likely that they have adapted to low fertility environments.

Previous studies have also shown that P response is significantly influenced by native soil fertility, soil texture and environmental factors affecting these conditions (Lorenz and Rogler, 1972). Soil tests showed that total soil P levels and availability were marginal to optimal (21-33ppm) for grass establishment in all soil types (Norwest Labs, 2000). The non-P-limiting conditions of the three soils may have reduced responses to additional P

fertilization (Olson et al., 1962). High levels of native P and conditions that reduce P adsorption, such as lower clay content and higher organic matter, increase the labile pool of P available for plant uptake (Sanchez and Uehara, 1980). Consequently, a high availability of native soil P may reduce the response to applied P (Barber, 1958). Future studies should be conducted on soils with low native P levels.

Previous studies have shown that *P. bilaii* inoculation increased P uptake, seedling vigor and biomass production of wheat, canola, and field bean (Kucey and Leggett, 1989; Downey and Van Kessel, 1990). These studies suggested that *P. bilaii* colonies form around the rhizosphere, solubolizing inorganic P and transforming it into forms available for plant uptake. However, recent studies have suggested that plant responses to *P. bilaii* are the result of increases in root mass and subsequent soil exploration, rather than increased P availability (Gulden and Vessey, 2000). Furthermore, *P. bilaii* responses are generally masked when soil P is not available in inorganic form, such as in soils with a high sand content or organic matter (Gleddie, 2001). The absence of response to *P. bilaii* treatments in this study may be the result of the relatively higher sand content of the three soil types. However, initial responses of some perennial crops to *P. bilaii* inoculation, including alfalfa, have not been observed for as long as 3 years after planting (Gleddie, 2001). It is reasonable to speculate that the duration of the experiment was too short to observe a response to the inoculant, which would occur when the existing labile pool of P has been expended.

Although soil texture influences soil fertility, previous studies have shown soil texture is more important in promoting the establishment and growth of certain native grass species than applying fertilizers (Power, 1980; Belanger et al., 2000). For example, a comparison between western wheatgrass and little bluestem showed that the infertile soils generally found on ridges are more likely to support warm-season (little bluestem) species than cool-season (western wheatgrass) (White, 1961). The SL soil type provided the most successful conditions for establishment and development of blue grama and prairie junegrass. Soil type was shown to be highly influential in determining the survival of the two grass species.

In conclusion, to ensure the successful establishment blue grama and prairie junegrass, each species should be planted in medium textured soils with good drainage; however, enough surface moisture to promote adventitious root development.

Determining distinctness and uniformity of blue grama [*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths] cultivars, ecotypes and ecovars™.

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ABSTRACT

Limited availability of adapted seed sources of native grasses has hindered their use in soil stabilization, habitat revegetation, and native rangeland seeding. The need for widely available and regionally adapted plant material resulted in the development of a new type of seed source called ecological varieties, or ecovars™. Ecovars™ are designed to improve stand establishment and seed production, while maintaining and/or improving plant and wildlife biodiversity by combining genetic diversity with improved plant characteristics (e.g. seedling vigor and seed production). Currently, it has not been determined if the genetic diversity within ecovars™ will allow them to fit the distinct, uniform and stable requirements necessary for Plant Breeders' Rights (PBR) protection.

The objective of this study was to determine the distinctness and uniformity of blue grama [*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths] cultivars, ecotypes and ecovars™. Vegetative and reproductive measurements were taken from a spaced-plant nursery planted in spring of 2000 in Carman, Manitoba, Canada and observed during 2000 and 2001. Results were analyzed and presented by year to accommodate the current PBR regulations, which require data from the establishment and the seed production years for perennial grasses. The ecovar™ was distinct from all pre-existing commercial populations included in this study (Bad River cultivar, Wyoming ecotype, Minnesota ecotype) over both years of production, and distinct from the original population (Manitoba-Multi-site) in the establishment year. The ecovar™ maintained a level of within-population

variability equal to its original population and to the Wyoming and Minnesota ecotypes, and showed more variability than the Bad River cultivar. These findings show that there is potential for the Manitoba blue grama ecovar™ to qualify for protection under the PBR Act of Canada.

INTRODUCTION

Native grasses, defined as those grass species indigenous to North America prior to European settlement, were the predominant vegetation in the North American Great Plains and formed the tall grass, mixed grass and short grass prairie regions. Blue grama [*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths], and prairie junegrass [*Koeleria macrantha* (Ledeb.) J.A. Schultes] are two predominant native short grass prairie species traditionally found throughout the Northern Great Plains of North America (Gould, 1975).

The drought and soil erosion problems of the 1930's stimulated interest in reclaiming the highly erodable lands on the Northern Great Plains of North America. Most revegetation efforts have focused on the use of introduced European and Eurasian grass species like tall fescue (*Festuca arundinacea* Schreb.), smooth bromegrass (*Bromus inermis* Leyss.) and crested wheatgrass (*Agropyron cristatum* L.) because of their ease of establishment and plentiful seed production (Jacobsen et al., 1994). However, studies over the last 30 years have shown that grass stands of many introduced species require regular replanting and maintenance, thereby increasing long-term costs (Duebbert et al., 1981). Previous studies have concluded that once established, native grasses are more tolerant to environmental extremes and require fewer long-term inputs (Jacobsen et al., 1994). Furthermore, traditional cultivars of introduced grass species have been developed for uniform growth and ease of harvest. The phenotypic uniformity of many introduced grass cultivars, in comparison to the

diversity found in remnant prairies and some native grass cultivars, has been linked to reductions in plant and wildlife biodiversity (Wilson and Belcher, 1989).

Consequently, reclamation and conservation plantings since the late 1970's have promoted the development and use of native grass cultivars rather than introduced grass cultivars (Jacobsen et al., 1994). However, successful establishment of many native grass cultivar plantings has been hindered due to their limited range of adaptability, often a result of their limited genotypic diversity or population variability (Allard, 1970; Jacobsen, 1984; Jacobsen et al., 1994).

Population variability within and among populations is maintained through annual exchanges of DNA via cross-pollination (Wipff, 1996) and through natural selection processes as they are influenced by climatic factors such as precipitation, temperature and daylength (Cooper, 1963), and soil related factors such as soil texture, soil fertility and soil depth (Dix and Butler, 1960). Population variability allows native grass stands to adapt to adverse conditions, including environmental extremes and disease and insect pressures. Previous studies have shown that habitat (e.g. soil type and precipitation and temperature patterns) is more influential in determining population variability in native grasses than geographic distance (Riegel, 1940; Cooper, 1963; Hamrick and Holden, 1979; Huff et al., 1993; Huff et al., 1998). Environmental influences can promote the development of distinct environmental adaptations for isolated populations (ecotypes) within a single species (Turesson, 1922a,b). As a result, the adaptability of native grass cultivars developed from locally adapted germplasm is

limited to regions with similar environmental conditions (Cooper, 1957; Allard, 1970; Jacobsen, 1984; Jacobsen et al., 1994).

Understanding and describing population variability becomes especially important when differentiating between two heterogeneous populations. For example, native grass populations tend to flower earlier, grow less vigorously, and produce less forage when grown in latitudes south of their original collection site (Savage, 1939; Duebbert et al., 1981; Pahl and Darroch, 1997). Consequently, plant growth and persistence of certain native species, including blue grama, prairie junegrass, and little bluestem, is compromised when they are grown in environments outside of their adaptive range.

Because of the limited range of adaptability of native grass cultivars, and the limited supplies of locally adapted plant material, a new type of seed source referred to as ecological varieties, or ecovars™, was developed. Ecovars™ were first suggested by Erling T. Jacobsen of the USDA-NRCS as a potential replacement for the native grass cultivar. The ecovar™ selection technique is designed to combine genetic diversity with improved plant characteristics (e.g. seed production and seedling vigor) (Jacobsen, 1984). Ideally, ecovars™ comprise equal geographical representation of all genetic material from all collection locations. As a result, each ecovar™ should be adapted to the range of environments from where it was originally collected (Phan and Smith, 1997a,b).

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). These criteria are often referred to as DUS (distinct, uniform and stable). Previous studies have shown that phenotypic and genotypic distinctness between native grass ecovars™ and their original populations is possible using agronomic traits such as seed and biomass production, culm heights and days to flowering, as well as DNA markers (Phan and Smith, 2000; Huff, 1997).

The objective of this study was to determine and compare the distinctness and uniformity of the blue grama ecovar™ to the Bad River cultivar, and Wyoming, Minnesota and Manitoba ecotypes. This study is intended to develop the methodology necessary to determine and compare distinctness and uniformity between ecovars™, cultivars and ecotypes that may potentially be applied to all native grasses. Genetic stability was not fully addressed in this study because only two generations of the ecovar™ were included in the examination.

MATERIALS AND METHODS

Population Histories

The plant materials included in this study were 1) two generations of the Manitoba ecovar™ (pre-breeder and breeder) and an unrelated original base

population, 2) the USDA released 'Bad River' cultivar, and 3) two commonly available privately developed ecotypes from Wyoming and Minnesota. The Bad River cultivar and Wyoming and Minnesota ecotypes were chosen because they are the primary northern adapted blue grama seed sources available in the Northern Great Plains, and may be considered most similar in morphology to the Manitoba ecovar™ (Smith, 2000).

The six populations were as follows:

1) Manitoba multi-site (MB-MS): is a seed population derived from the 1997 bulk seed harvest of the Original Population transplant nursery in Winnipeg, Manitoba, consisting of 495 wild blue grama plants collected from south-western Manitoba during the summer of 1992. This nursery consisted of 45 plants collected from each of 11 separate locations.
2) Ecovar pre-breeder (MB-PB): is seed harvested from a pre-breeder nursery established at the University of Manitoba, consisting of the 9 highest seed yielding plants from each of the 11 plant collections included in the Original Population represented in equal proportion. This was done to ensure that each of the collection gene pools was equally represented in this new population. The objective of the selection criteria was to maintain genetic diversity while improving seed yield and plant vigor. This ecovar™ development procedure is described in detail by Phan and Smith (2000).
3) Ecovar breeder (MBE): An equal amount of seed was collected from each of the 99 plants in the pre-breeder population. From each sample, at least 10

seedlings were established, and planted to form a 1000-plant breeder nursery.

4) Wyoming ecotype (WY): is a native seed collection of blue grama made by Clare Dunne from_Wind River Seeds, (Wind River Seeds, 3075 Lane 51 ½ Manderson, Wyoming, 82401, ph:(307) 568-3361, fax:(307) 568-3364) from the Wind River Range in western Wyoming. This population was increased in a commercial seed production field and is identified as a Wyoming ecotype.
5) Minnesota ecotype (MN): is a native seed collection of blue grama made by Oscar Carlson (Box 157, Lake Bronson, MN. 56734), a farmer from northern Minnesota. The collection was increased every year in a commercial seed production field and is identified as a northern Minnesota ecotype.
6) Bad River (BR): is a 'selected' class ecotype released in 1996 by the Natural Resource Conservation Service (NRCS) & Plant Materials Center (PMC) at Bismark, North Dakota (3308 University Dr., Bismarck, North Dakota 58504-7564). 'Bad River' originated from a single blue grama collection along the Bad River in North Dakota and increased in a commercial seed production field. NRCS Plant Material Center staff characterized this germplasm as having good seedling vigor, good growth characteristics and higher than average seed yields. This population was released as a 'selected' class ecotype under the Pre-Variety Germplasm Seed Certification program in the U.S. Bad River is the most widely distributed commercial blue grama seed source in the Northern Great Plains, but has been reported to exhibit limited diversity (Smith and Whalley, 2002).

Experimental Design

Field experiments were conducted in 2000 and 2001 at the University of Manitoba Carman Research Station in Carman, Manitoba, Canada. Transplants from the six populations of blue grama were grown from seed in greenhouse conditions in late February 2000, and transplanted using standard protocols to their outdoor location in early June 2000. The transplants were planted in a well-drained Eigenhof clay loam, which was previously tilled, and configured into a 27m x 23m grid, with 30cm distances between all plants to minimize plant interaction. The seed used for the MB-MS, MB-PB, and MBE populations was collected from previously established plots at the University of Manitoba. The seed for the MN and WY populations was obtained from retailers in their respective states, and the seed for the BR population was obtained from the NRCS-PMC in North Dakota. A Randomized Complete Block Design was used with 6 replications. Each replicate included 20 plants of each population, planted 2 rows wide and 10 plants long. Weed control consisted mainly of hand weeding, with periodic broadcast herbicide applications of Lontrel® at 0.84 L ha^{-1} (active ingredient: clopyralid – 360g/L) and ParIII® at 5.5 L ha^{-1} (active ingredients: mecoprop – 100g/L; 2-4,D – 190 g/L; dicamba – 18g/L) during heavy weed infestations, using a bicycle sprayer. Irrigation was used for the first 3 weeks after transplanting. Each plant was watered daily with monoammonium phosphate (11-52-0) applied at a 2% concentration of phosphorous to assist with root development and minimize transplant shock.

Measurements taken after plant growth ceased, and before plants became desiccated included: 1) flag leaf length, 2) flag leaf width, 3) height of tallest culm, 4) average culm height, 5) reproductive culm angle – the relative angle of the reproductive culms to the ground, 6) visual seed production score – individual plant score for relative seed production on a scale of 1–9 (1=lowest, 9=highest), 7) crown diameter and 8) spring dormancy – visual rating for vegetative growth on a scale of 1–5, with 5 representing the most vegetative growth across the nursery, taken when the majority of plants in the nursery showed at least some growth. Maturity scores were collected every 7 days, typically during the afternoon. Each plant was scored on the following scale: 1=vegetative, 2=booting, 3=heading, 4=flowering, 5=filling, 6=mature. Days to flowering was calculated from the date at which each plant achieved a score of 4, and converted to number of days after May 1, and was averaged for each population.

After maximum plant heights were achieved, five culms were randomly selected from each plant for detailed indoor measurements. These measurements included: 1) number of spikes per culm, 2) length of spike 1 – length of the highest (primary) spike, 3) length of spike 2 – length of the second highest (secondary) spike, 4) inter-spike length – the distance between spike 1 and 2, 5) first internode length – distance between the highest node on the culm and the lowest occurring spike, 6) spike weight – total weight of spikes collected in the 5-culm sample, and 7) culm node width – width of the highest node on the culm. After the growing season, all plants were hand harvested 7.5cm from ground level and dried at 80°C for 24 hours and weighed to determine biomass

production per plant. The 20 plants per treatment were combined and threshed to determine seed yield on a per plot basis.

Table 4.1. Characters measured on individual blue grama plants in 2000 and 2001 at the University of Manitoba field station in Carman Manitoba.

No.	Abbr.	Character	Character type[§]	Units	2000	2001
1	FLL	Flag leaf length	veg.	(mm)	x[†]	x
2	FLW	Flag leaf width	veg.	(mm	x	x
3	HTC	Height of tallest culm	veg.	(cm)	x	x
4	ACH	Average culm height	veg.	(cm)	x	x
5	RCA	Reproductive culm angle	veg.	(1-8)	x	x
6	DTF	Days to Flowering	reprod.	(days)	x	x
7	CNW	Culm node width	veg.	(mm)		x
8	FIL	First internode length	veg.	(mm)	x	x
9	LSP1	Length of spike 1 (primary)	reprod.	(mm)	x	x
10	LSP2	Length of spike 2 (secondary)	reprod.	(mm)	x	x
11	ISL	Inter-spike length	veg.	(mm)	x	x
12	NOSP	Average number of spikes	reprod.		x	x
13	SPWT	Total weight of spikes	reprod.	(g)	x	
14	VSP	Visual seed production score	reprod.	(1-9)		x
15	CD	Crown diameter	veg.	(cm)		x
16	PBP	Plant biomass production	veg.	(g)		x
17	SGU	Spring dormancy	veg.	(1-5)		x
18	SY	Seed yield[‡]	reprod.	(g)		x

[†] Denotes if a character was measured in a particular year.
[‡] Seed yield was measured on a per plot rather than per plant basis.
[§] Character type abbreviations: vegetative (veg.), reproductive (reprod.).

Analysis

Population Distinctness

Data for all variables were normally distributed in both years. Of the 12 and 17 characters measured in 2000 and 2001, respectively, 9 were measured in both years, and these were tested for year effects using the Analysis of Variance (ANOVA) procedure in SAS 8.02 (SAS Institute, 1988). The ANOVA procedure in SAS 8.02 was also used to separate means among populations for all characters within years.

The between-population discriminating power of each character was determined by calculating between- versus within-population variability ratios in each year using the four reference populations (WY, MN, BR, and MB-MS). The MB-PB and MBE were excluded to provide an unbiased examination of the distinctness between the ecovar™ and previously existing populations (WY, MN, BR, MB-MS). An ANOVA (SAS Institute, 1988) was used to calculate a mean square value (MS) for the population and the residual error term (EMS) for each character in each year. The population MS divided by the EMS produced an estimate of the ratio of between-population variability to within-population variability for each of the measured characters. Characters with the highest ratios provided the greatest amount of discriminating power between populations.

Canonical Discriminant Analysis (CDA) was used to calculate distinctness between the populations. Standardized data were used for the CDA analysis.

The data were standardized (S) as follows:

$S = Y_c / M_c$,

where Y_c is the measurement of a character for an individual plant, and M_c is the highest measurement recorded for that character in the experiment (Podani, 1994).

The WY, MN, BR, and MB-MS populations were also used as reference populations in the CDA analyses for determining the best characters for discrimination. Beginning with a CDA on the character ranked highest for its MS ratio, a discriminant analysis was conducted, with additional characters being added in each analysis until the CDA distance matrixes produced were correlated to the CDA distance matrixes produced after using all the characters measured. A Mantel matrix correlation (NTSYSpc v.2, 1986) was used to compare the CDA distance matrixes produced by each combination of characters to the CDA matrixes produced using all characters taken for each particular year. This approach was used to determine the minimum number of characters required to distinguish between the four reference populations. Ninety-nine per cent was used as the satisfactory correlation level between the Mantel comparisons. The set of characters determined for each year were used in CDA to calculate distance matrixes between the MBE and MB-MS, and between the MBE, BR, WY and MN.

Within Population Variability (Uniformity)

The description of population variability is important for identifying diversity within a population, in particular, a cultivar registered under the PBR Act in Canada. According to the PBR Act, any variation within a population/cultivar should be predictable to the extent that it can be described by the breeder, and be commercially acceptable. However, measuring population variability is a challenge (Hennink and Zeven, 1991). Previous studies have used and compared population variation indices (e.g. Shannon-Weaver diversity index, Nei's genetic diversity statistics) for their expediency and accuracy in measuring population phenotypic variability (Hutcheson, 1970; Hennink and Zeven, 1991). Some researchers suggest that these indices are often mathematically intensive, and their usefulness is subject to the readers' statistical background and interpretation (Hennink and Zeven, 1991; Phan, 2002). Hennink and Zeven (1991) concluded that there is no proper measure of population variability, and that perhaps the best measure of variability lies in the readers' interpretation of the statistics used. Therefore, coefficients of variation (CV) were chosen in this study to compare population variability because of their ease of calculation and common acceptance and understanding, while providing an accurate and relative assessment of population phenotypic variability (Sachs and Coulman, 1983).

The CV's were calculated via the ANOVA procedure in SAS 8.02 (SAS Institute, 1988). Coefficients of variation were calculated for each character for each population within each replicate and for each year.

The coefficient of variance (CV) was calculated as

$CV = \sigma_v / \bar{X} (100)$,

where σ_v is the standard deviation, and $\bar{X}$ the mean value of a given character measurement (Sachs and Coulman, 1983). Because coefficients of variation are unitless, they were averaged across characters within each of the 6 replicates, providing a total of 6 CV readings for each population. The ANOVA procedure in SAS 8.02 was used to separate the CV means among populations.

RESULTS AND DISCUSSION

Weather Data

Temperature and precipitation for the 2000 and 2001 growing seasons varied slightly from the 30-year average (Environment Canada, 2002) (Table 4.2). Temperatures were below average in both June and July 2000. Low temperatures, in conjunction with above normal precipitation in June (125% of average) may have delayed early spring growth of the blue grama populations and subsequently delayed flowering (Mitchell, 1953a,b; Ryle, 1966b,c). Below average precipitation in June and July 2001, in combination with warmer temperatures, may have been responsible for earlier flowering in the second growing season (Brown, 1943).

Population Distinctness

Seven of the eleven measured characters common to both years showed a year x treatment interaction. Data for each year was analyzed separately, and results presented for each year individually. This was justified because current PBR regulations require that measurements be made in both the establishment and seed production years for perennial grasses.

Table 4.2. Temperature and precipitation averages from May 2000 to September 2001, and 30-year averages for Carman, Manitoba, Canada.[†]

		Monthly Conditions		30 Year Average 1961-1990		Percent of 30 year average	
Year	Month	Mean T (°C)	Precip (mm)	Mean T (°C)	Precip (mm)	Temp (%)	Precip (%)
2000	May	11.5	55.0	11.6	54.1	0.99	1.02
	June	14.6	93.6	17.1	75.3	0.85	1.25
	July	18.9	46.8	19.8	77.2	0.96	0.61
	August	18.7	86.0	18.4	66.4	1.01	1.30
	September	11.8	40.0	12.5	49.3	0.95	0.82
	October	6.0	8.8	6.8	33.5	0.88	0.26
	November	-6.1	59.7	-3.5	22.2	1.74	2.69
	December	-20.9	30.7	-12.7	22.6	1.64	1.36
2001	January	-11.4	9.4	-16.0	20.1	0.71	0.46
	February	-17.2	9.9	-12.8	18.4	1.34	0.54
	March	-5.8	18.7	-5.5	28.1	1.05	0.67
	April	4.7	29.9	4.5	41.8	1.04	0.72
	May	12.8	52.6	11.6	54.3	1.10	0.97
	June	16.2	41.2	17.1	75.1	0.95	0.55
	July	19.8	192.5	19.8	77.3	1.00	2.50[‡]
	August	19.5	22.2	18.4	66.5	1.06	0.34
	September	13.4	13.6	12.5	49.2	1.07	0.28

[†]Source: Environment Canada Atmospheric Environment Service, Winnipeg, Manitoba. R3C 3V4

[‡] The majority of this precipitation occurred during a high rainfall event on July 26 & 27, when 141mm rain fell in a 24-hour period.

Ecovar™(MBE) vs. MB. multi-site population (MB-MS)

The CDA showed that the MBE population was highly distinct (p=0.0039) from the MB-MS population, a representative of the Original Population from which the MBE was selected, in the establishment year (2000) (Table 4.3). The Mantel test indicated that 10 of the 12 characters measured in the establishment year were required to accurately discriminate between the populations; the characters included were: days to flowering, flag leaf length and width, number of spikes per reproductive culm, first internode length, average culm height, reproductive culm angle, length between spikes 1 and 2, height of tallest culm, and spike weight (WY, BR, MB-MS, MN) (Table 4.4). However, on the basis of individual character mean comparisons, the two populations were not distinguishable, except on the basis of reproductive culm angle (Table 4.5). Differences between the MBE and MB-MS populations in the mean comparisons of the other characters were likely not found due to the relatively high amount of variability in the measured characters (Tables 4.5).

The MBE and MB-MS populations were indistinguishable in the seed production year (2001) according to the CDA (Table 4.6). The mean comparison did not show differences between the two populations for days to flowering and dry matter production (Tables 4.8a & b), which were the two characters confirmed by the Mantel test of correlation between distance matrixes to show distinctness between the reference populations (Table 4.7). Differences between the two populations were also not observed in the mean comparisons for any of the

Table 4.3: Canonical Discriminant Analysis (CDA) using the 10 characters determined by the Mantel test to discriminate between the testing populations (FLL, NOSP, FIL, FLW, ACH, RCA, HTC, DTF, ISL, SPWT)[f], 2000.

Population		Population	
Matrix 1[‡]	Wyoming	Bad River	MB. Multi-site
Wyoming	1		
Bad River	0.0001[†]	1	
MB. Multi-site	0.0001	0.0001	1
Minnesota	0.0001	0.0001	0.0024

Matrix 2[§]	Wyoming	Ecovar (Breeder)	Bad River
Wyoming	1		
Ecovar (Breeder)	0.0001	1	
Bad River	0.0001	0.0001	1
Minnesota	0.0001	0.0001	0.0001

Matrix 3[¶]	Ecovar (Breeder)	Ecovar (Prebreeder)
Ecovar (Breeder)	1	
Ecovar (Prebreeder)	0.0001	1
MB. Multi-site	0.0039	0.0001

[†] Significant at the 0.05 probability level.

[‡]CDA comparison between reference populations used for determining which characters were required.

[§]CDA comparison between the breeder generation of the ecovar™, Bad River, and Wyoming and Minnesota ecotypes.

[¶]CDA comparison between the breeder and prebreeder generations of the ecovar™, and the Manitoba multi-site population.

[f] Definitions of abbreviations: Flag leaf length (FLL), number of spikes (NOSP), first internode length (FIL), flag leaf width (FLW), average culm height (ACH), reproductive culm angle (RCA), height of tallest culm (HTC), days to flowering (DTF), inter-spike length (ISL), spike weight (SPWT).

Table 4.4: Mantel correlation values comparing Canonical Discriminant Analysis (CDA) distance matrixes created by using the combination of characters listed below versus a CDA distance matrix created using all characters measured, 2000.[†]

Character combinations[‡]	Mantel correlation value
FLL[§]	0.291
FLL, NOSP	0.586
FLL, NOSP, FIL	0.728
FLL, NOSP, FIL, FLW	0.709
FLL, NOSP, FIL, FLW, ACH	0.748
FLL, NOSP, FIL, FLW, ACH, RCA	0.820
FLL, NOSP, FIL, FLW, ACH, RCA, HTC	0.828
FLL, NOSP, FIL, FLW, ACH, RCA, HTC, DTF	0.824
FLL, NOSP, FIL, FLW, ACH, RCA, HTC, DTF, ISL	0.899
FLL, NOSP, FIL, FLW, ACH, RCA, HTC, DTF, ISL, SPWT	0.989
FLL, NOSP, FIL, FLW, ACH, RCA, HTC, DTF, ISL, SPWT, LSP2	0.991
FLL, NOSP, FIL, FLW, ACH, RCA, HTC, DTF, ISL, SPWT, LSP2, LSP1	1.000

[†] The four reference populations were used for comparing distance matrixes.
[‡] Combinations of characters were determined using the between:within variability ratios.
[§] Definitions of abbreviations: Flag leaf length (FLL), number of spikes (NOSP), first internode length (FIL), flag leaf width (FLW), average culm height (ACH), reproductive culm angle (RCA), height of tallest culm (HTC), days to flowering (DTF), inter-spike length (ISL), spike weight (SPWT), length of spike 2 (LSP2), length of spike 1 (LSP1).

Table 4.5: Means, Least Significant Differences and Coefficients of Variation of 12 characters measured on six blue grama populations in a spaced-plant nursery at the University of Manitoba field station in Carman, Manitoba, Canada, 2000.

		Measurements of Morphological Characters											
Population	Abbr.	Days to Flower	Flag Leaf Width	Flag Leaf Length	Height of Tallest Culm	Average Culm Height	Reproductive Culm Angle	First Internode Length[§]	Interspike Length[§]	No. Spikes per Culm[§]	Length Spike 1[§]	Length Spike 2[§]	Spike Weight[§]
		--days--[†]	--mm--	--mm--	--cm--	--cm--	(1 – 9)[¶]	--mm--	--mm--		--mm--	--mm--	--grams--
Wyoming Ecovar	(WY)	107.7*b*[‡]	1.35*e*	68.65*e*	43.30*a*	33.14*b*	5.2*ab*	211.42*a*	30.23*b*	1.6*d*	31.85*c*	28.77*c*	0.300*c*
(Breeder)	(MBE)	107.5*b*	1.61*a*	80.80*cd*	39.58*b*	29.97*c*	4.5*d*	190.70*cd*	27.95*cd*	1.9*b*	32.50*bc*	29.07*bc*	0.337*ab*
Bad River Ecovar	(BR)	110.2*ab*	1.58*ab*	106.25*a*	44.00*a*	34.90*a*	5.3*a*	199.97*b*	31.87*a*	1.8*c*	33.69*ab*	31.07*a*	0.355*a*
(Prebreeder)	(MB-PB)	94.9*c*	1.45*d*	78.22*d*	39.92*b*	29.96*c*	4.9*cd*	178.51*e*	27.17*d*	1.8*bc*	32.07*c*	28.96*bc*	0.288*c*
MB multi-site	(MB-MS)	108.0*b*	1.54*abc*	84.51*bc*	40.10*b*	30.99*c*	4.8*bc*	183.18*cde*	29.12*bc*	1.9*bc*	33.15*bc*	30.29*ab*	0.314*bc*
Minnesota	(MN)	112.0*a*	1.46*cd*	88.58*b*	40.22*b*	30.47*c*	4.5*d*	182.05*de*	29.44*bc*	2.0*a*	33.70*ab*	30.64*a*	0.331*ab*
LSD		2.84	0.083	6.02	2.14	1.72	0.33	9.21	1.56	0.08	1.44	1.51	0.028
CV		9.9	21.6	27.6	20.2	21.2	27.2	18.8	20.4	16.3	16.9	19.5	34.1

[†] Days to flowering was calculated as the number of days from May 1, 2000.
[‡] Means within columns followed by the same letters are not significantly different at p=0.05.
[§] Means were determined from 5 culm random samples.
[¶] Culm angle defined on a scale of 1-9, with 9 being vertical.

remaining 15 characters measured in 2001. Days to flowering may not have been different between MBE and MB-MS because of the level of accuracy for this score. Flowering data were collected every 10 days, with rating occurring during afternoons. Because an individual blue grama plant may flower for two weeks (Phan, 2002), the probability of rating a plant as flowering was good. However, the 10-day rating schedule would reduce the likelihood of rating a plant at the beginning of its flowering period. More accuracy for determining the start of flowering could be obtained if flowering data were collected daily, and preferably during the morning, before environmental factors (e.g. wind) remove anthers from the caryopses. The findings of this study do not agree with those of Phan and Smith (2000), who found that their blue grama original populations and ecovar™ progeny were distinct based on RAPD marker testing methods.

However, the seed bulking technique used to develop the MB-MS population must be considered when comparing between the MBE and MB-MS. The MB-MS population does not equally represent the individual collection sites of the original plant collections. The random seed sampling technique used to develop the MB-MS population would have adequately represented all original blue grama plant collections within the Original Population (Allard, 1970). However, the seed collected from the 1992 Original Population nursery was bulked over all plants and collections. By not accounting for variation within and between collections within the seed harvest, MB-MS would consist of higher proportions of robust and prolific seed producers. Therefore, the MB-MS population may not represent the Original Population because not all plant

Table 4.6: Canonical Discriminant Analysis (CDA) using the 2 characters determined by the Mantel test to discriminate between the testing populations (DTF, PBP)[f], 2001.

Population		Population	
Matrix 1[‡]	Wyoming	Bad River	MB. Multi-site
Wyoming	1		
Bad River	0.0001[†]	1	
MB. Multi-site	0.0001	0.0001	1
Minnesota	0.0001	0.0001	0.0001

Matrix 2[§]	Wyoming	Ecovar (Breeder)	Bad River
Wyoming	1		
Ecovar (Breeder)	0.0001	1	
Bad River	0.0001	0.0001	1
Minnesota	0.0001	0.0001	0.0001

Matrix 3[¶]	Ecovar (Breeder)	Ecovar (Prebreeder)
Ecovar (Breeder)	1	
Ecovar (Prebreeder)	0.3883	1
MB. Multi-site	0.4179	0.0435

[†] Significant at the 0.05 probability level.

[‡]CDA comparison between reference populations used for determining which characters were required.

[§]CDA comparison between the breeder generation of the ecovar™, Bad River, and Wyoming and Minnesota ecotypes.

[¶]CDA comparison between the breeder and prebreeder generations of the ecovar™, and the Manitoba multi-site population

[f] Definitions of abbreviations: Days to flowering (DTF), plant biomass production (PBP).

Table 4.7: Mantel correlation values comparing Canonical Discriminant Analysis (CDA) distance matrixes created by using the combination of characters listed below versus a CDA distance matrix created using all characters measured, 2001.[†]

Character combinations[‡]	Mantel correlation value
DTF[§]	0.741
DTF, PBP	0.986
DTF, PBP, FLL	0.976
DTF, PBP, FLL, ACH	0.987
DTF, PBP, FLL, ACH, VSP	0.996
DTF, PBP, FLL, ACH, VSP, SD	0.995
DTF, PBP, FLL, ACH, VSP, SD, CD	0.995
DTF, PBP, FLL, ACH, VSP, SD, CD, HTC	0.996
DTF, PBP, FLL, ACH, VSP, SD, CD, HTC, FIL	0.997
DTF, PBP, FLL, ACH, VSP, SD, CD, HTC, FIL, FLW	0.997
DTF, PBP, FLL, ACH, VSP, SD, CD, HTC, FIL, FLW, CNW	1.000

[†] The four reference populations were used for comparing distance matrixes.
[‡] Combinations of characters were determined using the between:within variability ratios.
[§] Definitions of abbreviations: Days to flowering (DTF), plant biomass production (PBP), flag leaf length (FLL), average culm height (ACH), visual seed production (VSP), spring dormancy (SD), crown diameter (CD), height of tallest culm (HTC), first internode length (FIL), inter-spike length (ISL), culm node width (CNW).

Table 4.8a: Means, Least Significant Differences and Coefficients of Variation of 17 characters measured on six blue grama populations in a spaced-plant nursery at the University of Manitoba field station in Carman, Manitoba, Canada, 2001.

		Measurements of Morphological Characters											
Population	Abbr.	Days to Flower	Flag Leaf Width	Flag Leaf Length	Height of Tallest Culm	Average Culm Height	Reprod-uctive Culm Angle	First Inter-node Length[§]	Inter-spike Length[§]	No. Spikes per Culm[§]	Length Spike 1[§]	Length Spike 2[§]	Culm Node Width[§]
		--days--[†]	--mm--	--mm--	--cm--	--cm--	(1 – 9)[¶]	--mm--	--mm--		--mm--	--mm--	--mm--
Wyoming Ecovar	(WY)	75.5*c*[‡]	1.53*cd*	62.75*d*	60.19*b*	51.75*b*	5.0*a*	281.29*ab*	37.48*a*	2.0*a*	37.71*b*	36.64*bc*	1.32*a*
(Breeder)	(MBE)	82.6*b*	1.58*bc*	73.41*c*	56.85*cd*	48.52*c*	4.6*a*	250.70*d*	35.31*c*	2.0*a*	36.95*b*	36.85*bc*	1.21*cd*
Bad River Ecovar	(BR)	82.4*b*	1.69*a*	93.67*a*	64.74*a*	56.89*a*	4.3*b*	284.90*a*	37.59*a*	2.0*a*	38.06*ab*	37.51*b*	1.31*a*
(Prebreeder)	(MB-PB)	82.0*b*	1.50*d*	66.97*d*	54.99*d*	46.48*d*	4.7*a*	234.22*e*	32.77*d*	2.0*a*	35.43*c*	35.66*c*	1.20*d*
MB multi-site	(MB-MS)	82.4*b*	1.57*bc*	75.47*c*	57.43*c*	49.05*c*	4.9*a*	260.80*cd*	35.78*bc*	2.0*a*	36.95*b*	37.20*b*	1.26*bc*
Minnesota	(MN)	87.4*a*	1.60*b*	74.85*c*	57.60*c*	49.74*c*	4.8*a*	256.30*d*	37.34*ab*	2.0*a*	37.53*b*	36.88*bc*	1.23*cd*
LSD		1.47	0.07	4.85	1.87	1.67	0.34	11.29	1.67	0.07	1.39	1.39	0.04
CV		6.7	15.7	24.6	14.8	15.6	27.6	15.9	17.9	13.7	14.2	14.6	12.3

[†] Days to flowering was calculated as the number of days from May 1, 2001.
[‡] Means within columns followed by the same letters are not significantly different at p=0.05.
[§] Means were determined from 5 culm random samples.
[¶] Culm angle defined on a scale of 1-9, with 9 being vertical.

Table 4.8b: Means, Least Significant Differences and Coefficients of Variation of 5 characters measured on six blue grama populations in a spaced-plant nursery at the University of Manitoba field station in Carman, Manitoba, Canada, 2001.:

		Measurements of Morphological Characters				
Population	Abbreviations	Crown Diameter	Plant Biomass	Spring Dormancy[‡]	Visual Seed Production Score[§]	Seed Production[¶]
		--cm--	--grams--	--(1-5)--	--(1-9)--	--grams--
Wyoming Ecovar	(WY)	11.53*c*[†]	76.07*f*	2.1*b*	6.2*d*	327.96*d*
(Breeder)	(MBE)	12.43*b*	99.39*de*	2.3*b*	6.9*bc*	425.58*bc*
Bad River Ecovar	(BR)	13.54*a*	159.40*a*	3.0*a*	7.1*ab*	552.02*a*
(Prebreeder)	(MB-PB)	12.26*b*	92.62*de*	2.3*b*	6.7*c*	345.68*cd*
MB multi-site	(MB-MS)	12.40*b*	106.65*cd*	2.3*b*	7.0*bc*	407.56*bcd*
Minnesota	(MN)	13.06*a*	118.66*b*	2.4*b*	7.4*a*	428.05*bc*
LSD		0.53	10.82	0.22	0.29	89.35
CV		15.5	38.5	33.9	15.2	18.1

[†] Means within columns followed by the same letters are not significantly different at p=0.05.
[‡] Spring dormancy measured on a scale of 1 – 5, with 5 representing the earliest initiation of spring growth
[§] Seed score was determined visually after grain fill on a scale of 1 – 9, 9 indicating prolific seed production.
[¶] Seed production was measured on a per plot basis, where all plants per population were combined in a replicate.

collections may have been equally represented. It would be more accurate to consider the MB-MS population as a population representing a range of native blue grama genotypes within Manitoba, with moderately improved seed production characteristics. Given the seed bulking technique, MB-MS should be more similar to MBE than to the Original Population.

Ecovar™ (MBE) vs. Bad River (BR) cultivar

The CDA distance matrix showed that the MBE was highly distinguishable from the BR population in the establishment year (p=.0001) (Table 4.3). When the characters were examined individually, differences between the MBE and BR populations were significant for seven of the ten selected characters as determined by the Mantel test (Table 4.4) in 2000. The MBE generally produced smaller plants than the BR, with character differences including shorter flag leaf lengths, first internode lengths, inter-spike lengths, average culm heights, and tallest culm heights than the BR population (Table 4.5). The majority of these differences could be attributed to the difference in daylength between the adaptive regions of the two populations (Riegel, 1940), which was in favor of the BR population. However, the BR population developed fewer spikes per culm, a relative measure of seed production (May et al., 1999), in the establishment year than the MBE. Previous studies have shown that native grasses, including blue grama, produce more biomass, taller culms, longer and wider flag leaves, fewer spikes and less seed when grown in latitudes north of their native habitats (Anderson and Aldous, 1938; Savage, 1939; Riegel, 1940).

The MBE appeared to be more prone to lodging in the establishment year, having more prostrate reproductive culms than the BR population (Table 4.5). However, the lower plant angle may be attributable to the higher amount of seed produced by the MBE.

Days to flowering has been shown to be a highly heritable trait, and an important characteristic for distinguishing between native grass populations (Benedict, 1940; Fisher et al., 1980; Knowles, 1987; Phan and Smith, 2000). Similar research has shown that blue grama populations originating from southern latitudes begin flowering later as they are grown in more northern latitudes (Savage, 1939; McMillan, 1959a,b). However, according to the mean comparisons, there were no differences between the MBE and BR populations in days to flowering in the establishment year of this study (Table 4.5). Days to flowering may not have been different between MBE and BR because of the low level of accuracy for this score.

The CDA distance matrix revealed that the MBE was highly distinguishable from the BR population in the second year ($p= 0.0001$) (Table 4.6). When the characters were examined individually via the mean comparisons, differences between the MBE and BR populations were significant for biomass, which was one of the two characters deemed most important for distinguishing them in 2001 (Table 4.8b). Previous studies have shown that days to flowering is a highly heritable trait, and an important characteristic for distinguishing between native grass populations (Benedict, 1940; Fisher et al., 1980; Knowles, 1987; Phan and Smith, 2000). However, as in the establishment year, days to flowering may not have been different because of the low level of accuracy for this measurement.

Further examination of the characters measured in 2001 revealed that flag leaf length and width, the first internode length, inter-spike length, average culm height and height of the tallest culm of the MBE were shorter than BR in the seed

production year (Table 4.8a). These findings coincide with previous population studies on blue grama showing that southern populations produced taller culms and more biomass when grown in northern locations (Anderson and Aldous, 1938; Savage, 1939).

In 2001, BR seed production was 30% higher than the MBE (Table 4.8b). Seed production data was not collected in 2000; however, if we compare the two populations using spike number and weight as relative measures of seed production (May et al., 1999), the BR would have produced less seed in 2000 than the MBE (Table 4.5). Similar variability in seed production over years has been observed in blue grama, and has been attributed to climatic conditions and population variability (Riegel, 1940; Phan and Smith, 2000). Previous research indicates that populations of southern origin generally produce less seed when grown in northern locations (Anderson and Aldous, 1938; Savage, 1939; Riegel, 1940, Phan and Smith, 2000). The superior seed production shown in the BR is attributable to its inherently higher seed yielding potential, which is a result of the deliberate selection which took place in the original collection, and non-deliberate selection which has taken place over the commercial seed increase generations.

The MBE was less prone to lodging than the BR population in the second year, having more erect reproductive culms (Table 4.8a). This is in contrast to the reproductive culm measurement taken in the establishment year, where the MBE was more prostrate than the BR population (Table 4.5). This change in reproductive culm angle from the establishment to seed production year may be attributable to the differences in response between the two populations to the

different weather patterns between years. Lower culm angles in blue grama can result from environmental factors such as high wind and abundant rainfall events, which increase the severity of lodging in blue grama (Phan, Personal Communication). However, the BR population showed lower culm angles than all the other populations studied in 2001 (Table 4.8a). As a result, it is reasonable to speculate that the superior seed production observed in the BR population, in comparison to the other populations, may have increased spike weights and incidences of lodging.

The BR population produced wider crown diameters than the MBE, which is associated with increased reproductive culm numbers and seed production (Table 4.8b) (Werner and Burton, 1991). This does not agree with previous research showing that blue grama crown diameter is negatively correlated with daylength, resulting in narrowing crown diameters as plants are grown in northern latitudes (Olmsted, 1943). Olmsted (1943) showed that crown diameters of blue grama populations originating from two regions, having 8 and 12-hour photoperiods, decreased as they were grown in a controlled environment under a 16 hour photoperiod.

The BR population initiated spring growth earlier in the seed production year in comparison to the MBE (Table 4.8b). The increased spring vigor may be a result of a reduced level of spring dormancy that is common to plants of southern latitudes.

Ecovar™ (MBE) vs. Wyoming (WY) and Minnesota (MN) ecotypes

The CDA distance matrix showed that the MBE was highly distinguishable from the WY and MN populations in both the establishment (2000) and seed production (2001) years ($p=0.0001$) (Tables 4.3 & 4.6). When the characters were examined individually, differences between the MBE and MN populations were significant for four of the ten selected characters in 2000 (Table 4.5) and the two selected characters as determined by the Mantel distance matrix correlation test in 2001 (Tables 4.8a & b). When days to flowering was averaged for each population, the MBE began flowering 5 days earlier than the MN population in both the establishment and seed production years. The MN population produced longer and wider flag leaves than the MBE in the establishment year, with no difference occurring in the seed production year. However, MN produced more dry matter in the seed production year. Riegel (1940) showed similar variability in flag leaf length and width measurements and biomass production between two years of evaluation in blue grama and attributed these differences to climatic conditions and population variability. No differences were detected between the MBE and MN populations in height of the tallest culm, average culm length and first internode length in either year of production. These findings agree with other population studies that showed native plants from southern sources flowered later and produced more biomass when grown in northern locations (Savage, 1939; McMillan, 1959a,b).

When days to flowering was averaged for each population, the MBE and WY populations flowered at the same time in the establishment year; however, the WY flowered 7 days earlier in the seed production year. This is in contrast to the findings of a study conducted by Riegel (1940), which showed that southern populations of blue grama flowered later when grown in northern latitudes, attributing the change in flowering dates to changes in daylength between the collection sites of the populations. However, the WY population originated from a more arid region approximately 1200 meters higher in altitude than the MBE, and overall, out of the 6 populations included in this study it was grown furthest from its native habitat. It is likely the WY initiated flowering earlier than the MBE because of differences in climatic conditions, including temperature, growing-degree-days, moisture availability, daylength (Jacobsen, 1984). These findings agree with a previous study conducted at the University of Manitoba, which showed that populations of blue grama from Texas and New Mexico began flowering earlier than all the northern populations that were included in the study (i.e. the Bad River cultivar and the Wyoming, Minnesota and Manitoba ecotypes) (Smith, unpublished data).

The MBE produced more spikes per reproductive culm and heavier spike weights than the WY population in the establishment year, and received a higher visual seed production score and seed yield in the second year of growth (Tables 4.5, 4.8a & b). However, according to Andersson (1995), later initiation of flowering reduces the time available for seed maturation before frost, and consequently affects seed production. The earlier initiation of anthesis of the WY

population in 2001 subjected the pollen to abnormally dry conditions (Table 4.2), thereby reducing successful fertilization and seed production in the second year (Table 4.8b). Poor seed set has been observed in several native grasses experiencing heat and xeric stress during flowering, including blue grama (Kilfoyle, 1995)

In comparison to the MN population, the MBE produced fewer spikes per culm and a shorter secondary spike length in the establishment year (Table 4.5). This coincides with previous studies showing that populations of blue grama from southern locations produced more and longer spikes when grown in northern locations (Riegel, 1940; Phan and Smith, 2000). However, in the second year of growth, the MBE population produced spike numbers and spike lengths comparable to the MN population.

The MBE produced plants with longer and wider flag leaves than the WY population, and shorter first internodal distances and inter-spike distances in both years of growth. The WY population was taller than the MBE population in both the establishment year and seed production years (Tables 4.5 & 4.8a & b). Previous studies have shown that blue grama populations produce taller plants when grown in locations north of their original habitat, attributed primarily to longer daylengths (Savage, 1939; Knowles, 1943; McMillan, 1959a,b).

The crown diameter of the WY population was narrower than that of the MBE population, which may be responsible for the lower biomass production in the WY population. This agrees with previous studies on blue grama, which have shown that a negative correlation exists between daylength and crown diameter,

as well as biomass production (Olmsted, 1943). Olmsted (1943) showed that crown diameters and biomass production of blue grama decreased as two populations, which originated from regions with 8 and 12-hour photoperiods, were grown in a controlled environment under a 16-hour photoperiod.

In contrast, the crown diameter of the MN population was wider than that of the MBE population and may be responsible for MN producing greater biomass than the MBE population (Werner and Burton, 1991). According to Olmsted (1943), we would expect the MN population to have a narrower crown diameter; however, the difference in daylength between the native habitat of the MN population and the location of this experiment may not be significant enough to provide a response in crown diameter.

Within-population Uniformity

The description of population uniformity is important for identifying variability within a population, in particular, a cultivar registered under the PBR Act in Canada. According to the PBR Act, any variation within a cultivar should be predictable to the extent that it can be described by the breeder, and be commercially acceptable.

Character x year interactions were not significant for the eleven characters measured in both years; therefore C.V.'s were combined across years.

Ecovar (MBE) vs. MB. multi-site population (MB-MS)

The mean comparisons of the CV's showed that the MBE had a level of phenotypic variability equal to that of the MB-MS population, which represented the Original Population from which it was selected (Table 4.9). This agrees with previous studies which showed that although distinct, derived populations of grass species maintained levels of genotypic variability equal to their original populations, including blue grama and little bluestem ecovars™ (Huff, 1997; Phan and Smith, 2000). Although the seed bulking technique used to develop the MB-MS population would favor a higher proportion of the prolific seed yielding genotypes within Manitoba compared to the Manitoba Original Population, a random sampling from the MB-MS population would more accurately represent the phenotypic variability existing in a Manitoba ecotype (Allard, 1970).

Table 4.9: Means and Least Significant Difference for coefficients of variation (CV) averaged across all characters in common for the 2 years for six blue grama populations grown in a spaced-plant nursery grown at the University of Manitoba field station in Carman, Manitoba, Canada, 2000 & 2001.

Population	Abbreviation	Coefficients of Variation
Ecovar (Prebreeder)	(MB-PB)	0.217 a†
Manitoba multi-site	(MB-MS)	0.204 b
Ecovar (Breeder)	(MBE)	0.194 bc
Wyoming	(WY)	0.192 c
Minnesota	(MN)	0.189 c
Bad River	(BR)	0.172 d
LSD		0.0116

† Means within columns followed by the same letters are not significantly different at p=0.05.

The population phenotypic variability increased during the initial selection stage of the MB-PB population (Table 4.9); however, the MB-PB population may have included more genetic diversity than that of the species' effective population size. Synchrony of flowering between plants within the ecovar™ nursery reduced the genetic diversity of the MBE to levels observed in the MB-MS population (Table 4.9).

Environmental factors and their effects on pollination strongly influence genetic shifts in cross-pollinating populations (Hamrick and Holden, 1979). Cross-pollinating populations with initially high levels of genotypic/phenotypic variability can adapt quickly to their environmental surroundings (Fisher et al., 1980). Fisher et al. (1980) showed that mean flowering dates in populations of *Stylosanthes humilis* (Townsville stylo) increased by six weeks within 10 years when moved to a latitude with longer daylengths. More recent studies have shown that once established, some cross-pollinating populations of grass species may increase their level of phenotypic diversity as they adapt to the edaphic and climatic conditions around them, sometimes surpassing the levels of phenotypic diversity observed in their original populations (Vaylay and van Santen, 2002; Rouf Mian et al., 2002). Given this maintenance of heterogeneity in cross-pollinating populations, it is more understandable why the level of variability within the MBE, although selected for improved seed production, is equal to that of the MB-MS, its original parent population.

Ecovar (MBE) vs. Bad River (BR), Wyoming (WY) and Minnesota (MN) populations

The limited phenotypic variability in BR compared to the MBE population may be linked to the origin of the BR germplasm. The BR population was developed from a single ecotype collected within a small geographic region. Although not having gone through a selection process, the original ecotype of the BR population was identified as having exceptional seedling vigor and higher than average seed yields than surrounding ecotypes (USDA, 1997). The ecovar™ selection criteria used for the MBE aimed to improve seed production compared to the Original Population; however, the selection process maintained equal geographical representation of all ecotypes from each of the collection locations. As a result, the MBE contained levels of within-population phenotypic variability higher than the BR population based on a comparison of the CV's (Table 4.9). Previous studies on blue grama population dynamics have shown distinct pockets of ecotypes as small as 8 hectares may exist as close as 100 meters apart (Harlan, 1958). The formation of these distinct pockets of genotypes/phenotypes has been linked to the influence of micro-environments with distinct temperature regimes, precipitation patterns and soil characteristics (Turesson, 1922a,b; Cooper, 1963; Huff et al., 1998). As a result, the gene pool used in the initial stages of development for the BR population may have been genetically limited.

Previous studies have shown the importance of within-population phenotypic variability in comparison to between-population phenotypic variability

(Hamrick and Godt, 1989), and the plasticity of within-population variability, especially when grown in heterogeneous environments (Vaylay and van Santen, 2002; Rouf Mian, et al., 2002). The present study showed that the BR population has relatively limited within-population phenotypic variability compared to the other populations included in the experiment. It is reasonable to speculate that because the BR population has limited within-population variability, the gene pool of the BR population includes relatively little genetic diversity.

In contrast, the CV mean comparison revealed that the MBE population had levels of phenotypic variability equal to both the WY and MN populations (Table 4.9). The WY and MN populations may have a level of within-population phenotypic variability higher than the BR population because of a number of factors, including wider collection zones and nonbiased selection criteria. Most populations of native grasses developed by private organizations have undergone little if any selection for improvement of agronomic characteristics (Young and Young, 1986). Although the exact range of collection sites and selection criteria used for the WY and MN populations was not reported, it is assumed that the experimental design used for the development of the breeder plots and seed increase fields was simple and unsophisticated enough to have included a wide range of within-population phenotypic variability (Smith, 2000).

SUMMARY AND CONCLUSIONS

This study characterizes the distinctness and within-population variability (uniformity) of six populations of blue grama in the Great Plains, and compares the populations to assess the potential for protection under the current Plant Breeders' Rights Act of Canada. The six populations of blue grama were planted at a single location in Carman, Manitoba, Canada in 2000, and observed during 2000 and 2001. This study examined the morphological distinctiveness between MBE, MB-MS, BR, WY and MN populations. In addition to distinctness, the levels of within-population phenotypic variability were compared between the populations.

Previous studies have demonstrated that significant levels of genetic control exist in important agronomic traits such as days to flowering, flag leaf length and width, plant height, spike length, number of spikes per plant, plant dry matter production and crown diameter (Anderson and Aldous, 1938; Sachs and Coulman, 1983; Werner and Burton, 1991). However, these traits also interact with a range of environmental factors, including precipitation, temperature, altitude, and soil type (Cooper, 1963; Hamrick and Holden, 1979; Knapp and Rice, 1996; Wipff, 1996; Huff et al., 1998). When phenotypes are influenced less by environmental conditions, changes in environmental conditions result in less phenotypic change. Flowering dates, flag leaf widths, number of spikes per culm, and anthocyanin coloration are examples of characteristics that are influenced less by environmental factors, compared to flag leaf length, plant height, biomass

production and seed production which respond to changes in the environment (e.g. soil fertility) (Anderson and Aldous, 1938; Riegel, 1940; Kneebone, 1957; Sachs and Coulman, 1983; Werner and Burton, 1991). Southern collections typically display later flowering dates, larger plants and reduced seed production when grown in northern latitudes. Breeding programs that use these traits as selection criteria have shown genetic distinctions between derived populations and their original populations, including the development of ecovars™ and some cultivars (Huff, 1997; Phan and Smith, 2000).

The objective of this study was to determine and compare the distinctness and uniformity of the blue grama ecovar™ to the Bad River cultivar, and Wyoming, Minnesota and Manitoba ecotypes. This study is also intended to develop the methodology necessary to determine and compare distinctness and uniformity between ecovars™, cultivars and ecotypes that may potentially be applied to all native grasses. Distinctness was measured using a Canonical Discriminant Analysis (CDA), a type of multivariate statistics, which has been used in taxonomic circles for distinguishing between populations for the past 30 years; however, has not been used for determining distinctness between populations being tested for protection under the PBR Act. This study determined that distinctions between the four blue grama reference populations (MB-MS, BR, WY, MN) could be made by using either ten out of the twelve characters measured in the establishment year (days to flowering, flag leaf length and width, number of spikes per reproductive culm, first internode length, average culm height, reproductive culm angle, length between spikes 1 and 2, height of tallest

culm, and spike weight) or two out of seventeen characters measured in the seed production year (dry matter production, days to flowering). Because one goal of this experiment was to determine the distinctness of the ecovar™ from the reference populations (WY, MN, BR, MB-MS), both generations of the ecovar™ (MBE, MB-PB) were not included in the initial character determining stages. Using the determined characters, the CDA showed distinctness between the MBE and the commercially available WY, MN, and BR populations. These characters were also used to determine that the MBE and MB-MS populations were distinct in the first year of growth, but not in the second year of growth. This may be a result of the genetic similarity of the two populations. It is recommended that further testing between the MBE and MB-MS populations include the analysis of more characters.

The MBE was shown to maintain a level of within-population phenotypic variability equal to the MB-MS population. This is supported by previous studies, which have shown that derived populations of perennial grasses, including ecovars™ and broadly developed cultivars, maintained similar levels of within-population genotypic and phenotypic variability compared to the original populations from which they were developed (Huff, 1997; Phan and Smith, 2000).

The MBE was shown to have a higher level of within-population phenotypic variability than the USDA-developed BR population, reflecting the different breeding criteria/methods used to develop these distinct populations. However, no differences of within-in population phenotypic variability were observed

between the MBE, WY and MN populations. This may reflect a similarity in the development of the three populations.

In conclusion, the ecovar™ was shown to be highly distinct from the other commercially available cultivars and ecotypes tested in this study, and to display reasonable levels of morphological variability in comparison to the WY and MN populations. We conclude that there is potential for the MBE to qualify for protection under the PBR Act of Canada. More testing is needed to determine if the MBE is distinct from the Original Population from which it was derived, and to determine if and when the shift in morphological variability observed between the prebreeder and breeder generations of the MBE begins to stabilize, and how this change in variability is related to the genetic stability of the ecovar™.

GENERAL DISCUSSION

The objectives of this study were to assess the effects of seeding rate, phosphorus availability, and soil texture on the establishment of blue grama and prairie junegrass ecovars™, and to determine the distinctness and uniformity of blue grama cultivars, ecotypes and ecovars™. Genetic stability was not fully addressed in this study because only two generations of the ecovar™ were included in the examination.

Many studies have concluded that native species have poor seedling vigor and are difficult to establish. Traditionally, poor seedling vigor has been attributed to poor germination and/or low fertility conditions. The results from the first experiment of this study show that establishment success of blue grama and prairie junegrass is strongly influenced by soil type and soil conditions. Blue grama and prairie junegrass populations are naturally found on eroded knolls with marginal soils under low fertility and moisture conditions. Their ability to function well under these adverse conditions may be the reason for the lack of response to the additions of fertilizer in this study.

More recent studies have shown that establishment failures of native grass stands may be due to the limited range of germplasm diversity used to develop many native grass cultivars. Due to a lack of availability, seed lots are often obtained from southern locations in the Great Plains for reclamation plantings in the northern Great Plains. Although some native grasses, including blue grama, exist in both extremes of the region, these populations are genotypically and

phenotypically distinct. Consequently, some recent studies support the use of locally adapted seed to increase establishment success for reclamation plantings. This practice is supported by the second experiment of this study, which showed that blue grama populations produced morphologically different plants when grown in an environment different than their native habitat. For example, the Wyoming ecotype produced relatively smaller and potentially less competitive plants compared to the more northern seed sourced populations. It is reasonable to conclude that when blue grama germplasm intended for reclamation planting is moved far out of its adaptive range, the result may be a short-lived native grass stand.

To address the issue of limited availability of locally adapted seed, ecovars™ have been developed as a replacement for unadapted native grass cultivars. In addition to being selected for improved seedling vigor and seed production, ecovars™ include a range of germplasm, which optimizes genetic diversity and increases the likelihood that a portion of the ecovar™ seed will be adapted to any given environment in which it is planted. However, in order for an ecovar to qualify for protection under the PBR Act of Canada, it must be shown to be Distinct (D) from all other commercially available cultivars within its species, Uniform (U) within its population or to display a predictable level of variability, and to be morphologically Stable (S) in successive generations. This study demonstrated that the blue grama Manitoba ecovar™ (MBE) is highly distinct from the commercially available populations included in this study (Bad River cultivar and Wyoming and Minnesota ecotypes). These are particularly useful

results, indicating that the MBE, although including a broad range of genotypes, is distinct, and can be phenotypically described for the purpose of PBR, without describing the entire species. However, the PBR Act may require that a native species ecovar™ or cultivar be distinct from the population from which it was derived. This study showed that the MBE was distinct from a representation of its original population (MB-MS) in the initial (establishment) year, but not in the second year. This difference between years is due to G (genotype) x E (environment) interactions, possibly stemming from a setback in growth from the transplant shock experienced by the plants in the initial year. An additional year of observation is recommended to determine if plant morphology stabilizes after the initial year of establishment.

Additionally, interpretation of these results should consider two points. First, the MB-MS does not truly represent the Original Population because of the bulking technique used during its development inadvertently selected for the more prolific seed producers versus a truly random sample of the population. Thus, it is recommended that future testing between ecovars™ and their original populations include an original population developed with equal amounts of seed from each of the plants included in the ecovar™ selection process. Second, this study attempted to develop general criteria, including the minimum number of characters required, for distinguishing between blue grama populations. The characters used for determining distinctness between the MBE and the MB-MS population were those required for determining distinctness between the MB-MS and the populations originating from collections made in the United States (BR,

WY, MN). Comparisons between populations from Manitoba, North Dakota, Minnesota and Wyoming, theoretically should not require as many characters to show distinctness as an evaluation between two populations originating from Manitoba. Therefore, further testing with more characters may show distinctness between the MBE and MB-MS populations. Without further evaluation, these results indicate that the MBE is not as phenotypically distinct from the MB-MS as it is from the US material. It is recommended that further analyses be conducted to determine the particular characters and the minimum number required to show distinctness between the MBE and MB-MS populations.

Within-population variability was also characterized for the studied populations. The ecovar™ displayed levels of phenotypic variability equivalent to the original population (MB-MS). These results show that ecovar™ development is able to maintain phenotypic diversity while selecting for improved characteristics. Within-population phenotypic diversity in the Bad River population was less than that of all other populations studied in this experiment. This was not unexpected considering the limited germplasm used in its development. The original seed source for Bad River is from one location and therefore genetic diversity and long-term adaptation across a range of environments may be limited.

In conclusion, this research showed that establishment rates of blue grama and prairie junegrass can be improved by increasing seeding rates, and more importantly by planting in course to medium textured soils. Ecovars™ may further increase establishment rates by providing a widely adapted seed source, of which

a portion of seed should be adapted to a range of reclamation sites. The blue grama ecovar™ showed positive signs of qualifying for protection under the PBR Act of Canada, as it was shown to be distinct from all other commercially available populations of blue grama. Additional years of observations may be required to determine if the variability of the ecovar™ is predictable and commercially acceptable, and if the ecovar™ is stable in successive generations. This case study provides a useful model that may be applicable for studying the distinctness between ecovars™ and cultivars and ecotypes of other native grasses.

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APPENDIX

Table A1. ANOVA results for blue grama establishment with 2 seeding rates, 4 phosphorus treatments, and three soil types in a controlled environment.

Table A2. ANOVA results for prairie junegrass establishment with 2 seeding rates, 4 phosphorus treatments, and three soil types in a controlled environment.

Table A3. Height, Leaf stage, and biomass production of blue grama and prairie junegrass as affected by soil type, seeding rate, P availability, and foliar N application, 8 weeks after planting.

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Table A5. ANOVA results for blue grama vigour with 2 seeding rates, 4 phosphorus treatments, two foliar nitrogen treatments, and three soil types in a controlled environment.

Table A6. ANOVA results for prairie junegrass vigour with 2 seeding rates, 4 phosphorus treatments, two foliar nitrogen treatments, and three soil types in a controlled environment.

Table A7. Effect of seeding rate, phosphorus fertilizer, fungal seed coating, and tillage on establishment, plant height and leaf stage accumulation of blue grama grown at the University of Manitoba field station in Carman, Manitoba, 2000 and 2001.

Table A8. Effect of seeding rate, phosphorus fertilizer, fungal seed coating, and tillage on emergence, height and leaf stage development of prairie junegrass grown at the University of Manitoba field station in Carman, Manitoba, 2000 and 2001.

Table A9. ANOVA results for blue grama with two seeding rates, three phosphorus treatments and two tillage treatments grown at the University of Manitoba field station in Carman, Manitoba, 2000 and 2001.

Table A10. ANOVA results for prairie junegrass with two seeding rates, three phosphorus treatments and two tillage treatments grown at the University of Manitoba field station in Carman, Manitoba, 2000 and 2001.

Table A11.Mean square ratios of between-population vs. within-population variability for morphological characteristics measured in the reference blue grama populations (WY, MN, BR, MB-MS) grown at the University of Manitoba field station in Carman, Manitoba, 2000.

Table A12. Mean square ratios of between-population vs. within-population variability for morphological characteristics measured in the four blue grama populations (WY, MN, BR, MB-MS) grown at the University of Manitoba field station in Carman, Manitoba, 2001.

Table A13. ANOVA results for the mean separations of 12 characters measured in 6 blue grama populations grown at the University of Manitoba field station in Carman, Manitoba, 2000.

Table A14. ANOVA results for the mean separations of 17 characters measured in 6 blue grama populations grown at the University of Manitoba field station in Carman, Manitoba, 2001.

Table A15. ANOVA results for the mean separations of the coefficients of variation for the 6 blue grama populations combined across years, grown at the University of Manitoba field station in Carman, Manitoba, 2000 and 2001.

Table A1. ANOVA results for blue grama establishment with 2 seeding rates, 4 phosphorus treatments, and three soil types in a controlled environment.

Emergence. (R2=.805 CV=39.6)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Soil	2	57040.88	28520.44	12.24	.0002
Seedrate	1	11515.93	11515.93	4.94	0.036
Fert	3	7112.62	2370.87	1.02	0.402
Soil*Seedrate	2	1383.28	691.64	0.30	0.746
Soil*Fert	6	6409.50	1068.25	0.46	0.832
Seedrate*Fert	3	3643.31	1214.44	0.52	0.672
Soil*Seedrate*Fert	6	9487.33	1581.22	0.68	0.668
Rep(Soil*Seedrate*Fert)	24	55915.70	2329.82	15.51	<.0001
Day	11	18694.55	1699.50	11.31	<.0001
Soil*day	17	11397.72	670.45	4.46	<.0001
Seedrate*day	11	404.73	36.79	0.24	0.994
Fert*day	33	9656.99	292.64	1.95	0.001
Soil*Seedrate*day	17	262.36	15.43	0.10	1.000
Soil*Fert*day	51	9527.27	186.81	1.24	0.125
Seedrate*Fert*day	33	821.09	24.88	0.17	1.000
Soil*Seedrate*Fert*day	51	1186.59	23.27	0.15	1.000

Height. (R2=.925 CV=9.03)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Soil	2	9.11	4.56	5.63	0.010
Seedrate	1	0.01	0.01	0.02	0.896
Fert	3	3.54	1.18	1.46	0.250
Soil*Seedrate	2	0.06	0.03	0.04	0.963
Soil*Fert	6	6.80	1.13	1.40	0.255
Seedrate*Fert	3	0.81	0.27	0.33	0.802
Soil*Seedrate*Fert	6	4.72	0.79	0.97	0.465
Rep(Soil*Seedrate*Fert)	24	19.40	0.81	7.66	<.0001
Day	11	700.13	63.65	603.32	<.0001
Soil*day	17	14.13	0.83	7.88	<.0001
Seedrate*day	11	1.13	0.10	0.97	0.471
Fert*day	33	7.94	0.24	2.28	<.0001
Soil*Seedrate*day	17	0.90	0.05	0.50	0.954
Soil*Fert*day	51	5.20	0.10	0.97	0.543
Seedrate*Fert*day	33	2.02	0.06	0.58	0.972
Soil*Seedrate*Fert*day	51	3.60	0.07	0.67	0.963

Leaf stage. (R2= .899 CV=19.4)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Soil	2	1.08	0.54	3.74	0.039
Seedrate	1	0.02	0.02	0.11	0.738
Fert	3	0.19	0.06	0.45	0.719
Soil*Seedrate	2	0.31	0.16	1.08	0.356
Soil*Fert	6	1.36	0.23	1.58	0.198
Seedrate*Fert	3	0.39	0.13	0.90	0.457
Soil*Seedrate*Fert	6	1.78	0.30	2.06	0.096
Rep(Soil*Seedrate*Fert)	24	3.45	0.14	1.97	0.004
Day	11	320.10	29.10	398.86	<.0001
Soil*day	16	9.30	0.58	7.97	<.0001
Seedrate*day	11	0.21	0.02	0.26	0.992
Fert*day	33	3.02	0.09	1.25	0.158
Soil*Seedrate*day	16	0.56	0.03	0.48	0.959
Soil*Fert*day	48	5.96	0.12	1.70	0.003
Seedrate*Fert*day	33	3.59	0.11	1.49	0.040
Soil*Seedrate*Fert*day	48	3.47	0.07	0.99	0.492

Table A2. ANOVA results for prairie junegrass establishment with 2 seeding rates, 4 phosphorus treatments, and three soil types in a controlled environment.

Emergence. (R2= .885 CV=29.8)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Soil	2	144062.79	72031.40	13.86	<.0001
Seedrate	1	17526.31	17526.31	3.37	0.079
Fert	3	5283.72	1761.24	0.34	0.797
Soil*Seedrate	2	17543.12	8771.56	1.69	0.206
Soil*Fert	6	35389.93	5898.32	1.14	0.372
Seedrate*Fert	3	9989.90	3329.97	0.64	0.596
Soil*Seedrate*Fert	6	19769.93	3294.99	0.63	0.702
Rep(Soil*Seedrate*Fert)	24	124693.63	5195.57	23.68	<.0001
Day	11	89737.17	8157.92	37.19	<.0001
Soil*day	14	5292.64	378.05	1.72	0.047
Seedrate*day	11	1619.52	147.23	0.67	0.767
Fert*day	33	9822.24	297.64	1.36	0.091
Soil*Seedrate*day	14	230.78	16.48	0.08	1.000
Soil*Fert*day	42	1425.55	33.94	0.15	1.000
Seedrate*Fert*day	33	1041.73	31.57	0.14	1.000
Soil*Seedrate*Fert*day	42	2330.27	55.48	0.25	1.000

Height. (R2=.936 CV=7.34)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Soil	2	10.98	5.49	14.99	<.0001
Seedrate	1	0.43	0.43	1.17	0.289
Fert	3	1.37	0.46	1.24	0.316
Soil*Seedrate	2	0.34	0.17	0.47	0.631
Soil*Fert	6	3.09	0.52	1.41	0.252
Seedrate*Fert	3	0.88	0.29	0.80	0.507
Soil*Seedrate*Fert	6	0.86	0.14	0.39	0.877
Rep(Soil*Seedrate*Fert)	24	8.79	0.37	5.98	<.0001
Day	11	490.76	44.61	728.88	<.0001
Soil*day	15	9.54	0.64	10.39	<.0001
Seedrate*day	11	0.45	0.04	0.66	0.775
Fert*day	33	4.30	0.13	2.13	0.000
Soil*Seedrate*day	15	0.96	0.06	1.05	0.405
Soil*Fert*day	45	2.01	0.04	0.73	0.906
Seedrate*Fert*day	33	3.87	0.12	1.91	0.002
Soil*Seedrate*Fert*day	45	1.56	0.03	0.57	0.991

Leaf stage. (R2= .927 CV=20.39)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Soil	2	6.79	3.39	17.87	<.0001
Seedrate	1	0.02	0.02	0.09	0.767
Fert	3	0.91	0.30	1.60	0.216
Soil*Seedrate	2	0.34	0.17	0.90	0.418
Soil*Fert	6	0.61	0.10	0.54	0.774
Seedrate*Fert	3	0.45	0.15	0.80	0.508
Soil*Seedrate*Fert	6	1.15	0.19	1.01	0.443
Rep(Soil*Seedrate*Fert)	24	4.56	0.19	2.71	<.0001
Day	11	350.13	31.83	454.30	<.0001
Soil*day	14	5.30	0.38	5.40	<.0001
Seedrate*day	11	0.53	0.05	0.68	0.754
Fert*day	33	4.20	0.13	1.82	0.004
Soil*Seedrate*day	14	0.87	0.06	0.88	0.577
Soil*Fert*day	42	2.60	0.06	0.88	0.682
Seedrate*Fert*day	33	2.98	0.09	1.29	0.134
Soil*Seedrate*Fert*day	42	1.94	0.05	0.66	0.952

Table A3. Height, Leaf stage, and biomass production of blue grama and prairie junegrass as affected by soil type, seeding rate, P treatment, and foliar N application, 8 weeks after planting.

Treatment	Height (mm)		Leaf stage		Biomass (g)	
	Blue grama	Prairie junegrass	Blue grama	Prairie junegrass	Blue grama	Prairie junegrass
Soil Type	**	**	***	***	***	***
Sandy clay loam	132.1*c*[†]	52.7*b*	16.6*b*	16.8*b*	n/a	n/a
Sandy loam	260.7*a*	81.1*a*	35.7*a*	35.7*a*	0.518*a*	0.478*a*
Loamy Sand	217.7*b*	59.5*b*	34.8*a*	39.5*a*	0.344*b*	0.274*b*
LSD	25.18	7.26	9.6	5.2	0.171	0.139
Seed rate	ns[‡]	ns	ns	ns	ns	ns
100 seeds m^{-1}	206.8	67.7	30.1	33.1	0.419	0.384
200 seeds m^{-1}	191.8	64.1	28.0	31.5	0.469	0.377
LSD	16.95	5.24	4.3	4.2	0.127	0.074
P treatment	ns	ns	ns	ns	ns	ns
0 kg ha^{-1}	188.2	64.3	25.9	27.2	0.307	0.390
33 kg ha^{-1}	211.1	65.4	31.4	36.1	0.523	0.403
66 kg ha^{-1}	207.0	65.3	33.2	31.0	0.527	0.364
P. bacilli	191.2	68.3	25.8	34.7	0.398	0.366
LSD	23.98	7.4	6.08	9.21	0.180	0.104
Foliar N	ns	ns	**[§]	ns	ns	ns
N-	199.1	65.8	27.0	32.3	0.449	0.399
N+	200.0	62.8	27.7	32.2	0.436	0.362
LSD	15.76	3.48	4.16	3.29	2.04	0.066

[†] Means within columns followed by the same letters are not significantly different.
[‡] ns = not significant.
[§] interactions occurred between foliar N x seeding rate and foliar N x soil type. The separated means are shown in table A4.
** Significant at the 0.05 probability level.

Table A4. Blue grama leafstage as affected by foliar N x soil type and foliar N x seeding rate interactions 8 weeks after planting in a controlled environment.

Foliar N treatment	Soil type			Seeding rate	
	Sandy clay loam	Sandy loam	Loamy sand	100 seeds m^{-1}	200 seeds m^{-1}
N-	12.5[†] (9.6)[‡]	35.4 (10.9)	36.7 (22.8)	25.0 (14.1)	29.2 (22.5)
N+	15.5 (11.1)	36.0 (11.8)	32.6 (20.9)	31.6 (17.9)	23.5 (15.4)

[‡] numbers in brackets are standard deviations.
[†] means were not compared for significant differences.

Table A5. ANOVA results for blue grama vigour with 2 seeding rates, 4 phosphorus treatments, two foliar nitrogen treatments, and three soil types in a controlled environment.

Height. (R2= .686 CV=6.97)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Soil	2	1.87	0.93	6.31	0.006
Seedrate	1	0.00	0.00	0.03	0.856
Fert	3	0.32	0.11	0.73	0.544
Soil*Seedrate	2	0.12	0.06	0.42	0.664
Soil*Fert	6	0.31	0.05	0.35	0.905
Seedrate*Fert	3	1.02	0.34	2.31	0.102
Soil*Seedrate*Fert	6	0.95	0.16	1.08	0.404
Rep(Soil*Seedrate*Fert)	24	3.55	0.15	1.11	0.354
FoliarN	1	0.05	0.05	0.38	0.541
Soil*FoliarN	2	0.29	0.15	1.09	0.341
Seedrate*FoliarN	1	0.39	0.39	2.92	0.091
Fert*FoliarN	3	0.29	0.10	0.73	0.535
Soil*Seedrate*FoliarN	2	0.06	0.03	0.24	0.789
Soil*Fert*FoliarN	6	0.52	0.09	0.64	0.695
Seedrate*Fert*FoliarN	3	0.03	0.01	0.08	0.969
Soil*Seedrate*Fert*FoliarN	6	0.56	0.09	0.70	0.648

Leaf stage. (R2= .688 CV=14.12)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Soil	2	5.18	2.59	10.55	0.001
Seedrate	1	0.01	0.01	0.04	0.839
Fert	3	0.64	0.21	0.86	0.474
Soil*Seedrate	2	0.46	0.23	0.94	0.406
Soil*Fert	6	0.97	0.16	0.66	0.681
Seedrate*Fert	3	0.72	0.24	0.97	0.421
Soil*Seedrate*Fert	6	1.47	0.25	1.00	0.447
Rep(Soil*Seedrate*Fert)	24	5.89	0.25	1.17	0.289
FoliarN	1	0.00	0.00	0.02	0.879
Soil*FoliarN	2	1.56	0.78	3.72	0.028
Seedrate*FoliarN	1	0.82	0.82	3.93	0.051
Fert*FoliarN	3	0.76	0.25	1.21	0.310
Soil*Seedrate*FoliarN	2	0.35	0.17	0.84	0.437
Soil*Fert*FoliarN	6	1.05	0.17	0.83	0.547
Seedrate*Fert*FoliarN	3	0.21	0.07	0.34	0.795
Soil*Seedrate*Fert*FoliarN	6	0.66	0.11	0.53	0.786

Biomass production. (R2=.774 CV=63.7)

Source	DF	TypeIII SS	Mean Square	F Value	Pr > F
Soil	1	0.50	0.50	4.72	0.035
Seedrate	1	0.04	0.04	0.39	0.534
Fert	3	0.91	0.30	2.88	0.047
Soil*Seedrate	1	0.25	0.25	2.33	0.134
Soil*Fert	3	0.10	0.03	0.32	0.811
Seedrate*Fert	3	0.23	0.08	0.71	0.549
Soil*Seedrate*Fert	3	0.16	0.05	0.49	0.689
Rep(Soil*Seedrate*Fert)	45	4.75	0.11	1.33	0.212
FoliarN	1	0.02	0.02	0.22	0.643
Soil*FoliarN	1	0.01	0.01	0.08	0.783
Seedrate*FoliarN	1	0.03	0.03	0.39	0.536
Fert*FoliarN	3	0.08	0.03	0.33	0.807
Soil*Seedrate*FoliarN	1	0.00	0.00	0.04	0.843
Soil*Fert*FoliarN	3	0.26	0.09	1.11	0.362
Seedrate*Fert*FoliarN	3	0.00	0.00	0.01	0.999
Soil*Seedrate*Fert*FoliarN	3	0.11	0.04	0.47	0.707

Table A6. ANOVA results for prairie junegrass vigour with 2 seeding rates, 4 phosphorus treatments, two foliar nitrogen treatments, and three soil types in a controlled environment.

Height. (R2= .646 CV=4.76)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Soil	2	1.33	0.67	11.41	.0002
Seedrate	1	0.06	0.06	1.10	0.305
Fert	3	0.22	0.07	1.26	0.310
Soil*Seedrate	2	0.13	0.06	1.11	0.346
Soil*Fert	6	0.09	0.01	0.25	0.956
Seedrate*Fert	3	0.03	0.01	0.17	0.915
Soil*Seedrate*Fert	6	0.27	0.05	0.78	0.596
Rep(Soil*Seedrate*Fert)	23	1.34	0.06	1.53	0.078
FoliarN	1	0.02	0.02	0.51	0.477
Soil*FoliarN	2	0.12	0.06	1.57	0.214
Seedrate*FoliarN	1	0.01	0.01	0.28	0.598
Fert*FoliarN	3	0.01	0.00	0.11	0.956
Soil*Seedrate*FoliarN	2	0.04	0.02	0.47	0.627
Soil*Fert*FoliarN	6	0.15	0.02	0.64	0.697
Seedrate*Fert*FoliarN	3	0.09	0.03	0.78	0.510
Soil*Seedrate*Fert*FoliarN	5	0.09	0.02	0.49	0.783

Leaf stage. (R2= .773 CV=10.66)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Soil	2	1.91	0.96	3.77	0.038
Seedrate	1	0.00	0.00	0.01	0.931
Fert	3	0.46	0.15	0.61	0.615
Soil*Seedrate	2	0.71	0.36	1.41	0.263
Soil*Fert	6	1.45	0.24	0.96	0.474
Seedrate*Fert	3	0.84	0.28	1.11	0.365
Soil*Seedrate*Fert	6	2.10	0.35	1.38	0.262
Rep(Soil*Seedrate*Fert)	24	6.07	0.25	1.95	0.013
FoliarN	1	0.01	0.01	0.08	0.776
Soil*FoliarN	2	0.26	0.13	0.99	0.376
Seedrate*FoliarN	1	0.30	0.30	2.31	0.132
Fert*FoliarN	3	0.29	0.10	0.73	0.535
Soil*Seedrate*FoliarN	2	0.11	0.06	0.43	0.650
Soil*Fert*FoliarN	6	0.80	0.13	1.03	0.412
Seedrate*Fert*FoliarN	3	0.28	0.09	0.72	0.543
Soil*Seedrate*Fert*FoliarN	6	0.13	0.02	0.17	0.985

Biomass production. (R2= .483 CV=48.1)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Soil	1	0.56	0.56	14.97	0.001
Seedrate	1	0.01	0.01	0.21	0.655
Fert	3	0.10	0.03	0.86	0.481
Soil*Seedrate	1	0.03	0.03	0.87	0.364
Soil*Fert	3	0.20	0.07	1.79	0.190
Seedrate*Fert	3	0.06	0.02	0.51	0.678
Soil*Seedrate*Fert	3	0.13	0.04	1.17	0.352
Rep(Soil*Seedrate*Fert)	16	0.60	0.04	1.11	0.359
FoliarN	1	0.03	0.03	0.92	0.341
Soil*FoliarN	1	0.00	0.00	0.14	0.714
Seedrate*FoliarN	1	0.01	0.01	0.18	0.675
Fert*FoliarN	3	0.02	0.01	0.19	0.902
Soil*Seedrate*FoliarN	1	0.01	0.01	0.32	0.572
Soil*Fert*FoliarN	3	0.03	0.01	0.29	0.835
Seedrate*Fert*FoliarN	3	0.00	0.00	0.02	0.997
Soil*Seedrate*Fert*FoliarN	3	0.02	0.01	0.19	0.902

Table A7. Effect of seeding rate, phosphorus fertilizer, fungal seed coating, and tillage on establishment, plant height and leaf stage accumulation of blue grama grown at the University of Manitoba field station in Carman, Manitoba, 2000 and 2001.

	2000[†]			2001		
Treatment	Establishment	Height	Leaf stage	Establishment	Height	Leaf stage
Seeding rate						
	ns[‡]	ns	ns	ns	ns	ns
100 seeds m^{-1}	6.6*a*[§]	13.3a	22.9a	8.2a	229.5a	60.1a
200 seed m^{-1}	6.5*a*	13.7a	23.7a	11.3a	268.2a	76.2a
LSD	2.57	4.93	8.71	3.25	42.85	18.4
P treatment						
	ns	ns	ns	ns	ns	ns
0 kg ha-1 P	6.5a	14.3a	24.8a	7.7a	220.8a	58.6a
33 kg ha-1 P	5.5a	11.5a	20.8a	10.4a	240.4a	69.9a
Penicillium bilaii	7.7a	14.7a	24.4a	11.2a	286.7a	76.5a
LSD	3.14	6.02	10.6	2.44	78.26	2.44
Tillage system						
	**	**	**	ns	ns	ns
Conventional till	7.8a	15.6a	26.3a	9.9a	254.2a	65.9a
Zerotill	2.1b	5.52b	12.5b	9.7a	244.6a	70.6a
LSD	2.19	3.33	11.8	3.95	61.78	20.8

[†] 2000 experience extremely poor emergence and establishment conditions due to heavy spring rains and flooding in various regions of the plots. Zerotill plots suffered the most due to excess moisture.

[‡] ns = not significant.

[§] Means within columns followed by the same letters are not significantly different

** Significant at the 0.05 probability level.

Table A8. Effect of seeding rate, phosphorus fertilizer, fungal seed coating, and tillage on emergence, height and leaf stage development of prairie junegrass grown at the University of Manitoba field station in Carman, Manitoba, 2000 and 2001.[w].

	2000			2001[†]		
Treatment	Establishment	Height	Leaf stage	Establishment	Height	Leaf stage
Seeding rate						
	ns‡	ns	ns	ns	ns	ns
100 seeds m^{-1}	3.2a[§]	4.3a	7.3a	2.9a	50.7a	8.7a
200 seed m^{-1}	4.4a	5.0a	8.1a	4.5a	59.7a	11.8a
LSD	1.77	1.16	2.01	1.82	14.72	3.59
P treatment						
	ns	ns	ns	ns	ns	ns
0 kg ha-1 P	5.1a	4.4a	7.8a	4.9a	58.9a	11.3a
33 kg ha-1 P	3.4a	5.3a	9.1a	3.3a	49.6a	10.5a
Penicillium bilaii	3.1a	4.3a	6.1a	2.7a	58.8a	9.1a
LSD	2.17	1.42	5.23	2.81	18.09	3.6
Tillage system						
	**	ns	**	ns	ns	ns
Conventional till	5.1a	4.5a	8.4a	2.5a	44.9a	6.8a
Zerotill	2.3b	4.9a	6.9b	4.7a	63.3a	12.9a
LSD	1.56	0.58	1.35	3.01	18.85	8.23

[†]2001 experienced poor emergence and establishment conditions due to flooding in various regions of the plots.
‡ ns = not significant.
§ Means within columns followed by the same letters are not significantly different.
** Significant at the 0.05 probability level.

Table A9. ANOVA results for blue grama with two seeding rates, three phosphorus treatments and two tillage treatments grown at the University of Manitoba field station in Carman, Manitoba, 2000 and 2001.

Emergence, 2000. (R2= .886 CV=32.2)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	64.11	21.37	4.81	0.005
Seedrate	1	0.06	0.06	0.00	0.967
Fert	2	9.38	4.69	0.14	0.871
Rep*Seedrate	3	86.97	28.99	6.53	0.001
Rep*Fert	6	283.44	47.24	10.64	<.0001
Rep*Seedrate*Fert (Error 1)	6	199.48	33.25	7.49	<.0001
Tillage	1	171.44	171.44	11.51	0.019
Seedrate*Tillage	1	18.46	18.46	4.16	0.046
Fert*Tillage	2	41.19	20.59	4.64	0.014
Seedrate*Fert*Tillage	1	45.88	45.88	3.08	0.140
Rep*Seedrate*Fert*Tillage (Error 2)	5	74.49	14.90	3.36	0.010
day	3	23.99	8.00	1.80	0.158
Seedrate*day	3	7.32	2.44	0.55	0.651
Fert*day	6	6.80	1.13	0.26	0.955
Tillage*day	3	14.06	4.69	1.06	0.375
Seedrate*Fert*Tillage*day	17	32.87	1.93	0.44	0.970

Emergence, 2001. (R2= .919 CV=24.5)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	1228.42	409.47	71.04	<.0001
Seedrate	1	267.34	267.34	4.20	0.086
Fert	2	404.38	202.19	3.18	0.115
Rep*Seedrate	3	369.56	123.19	21.37	<.0001
Rep*Fert	6	741.79	123.63	21.45	<.0001
Rep*Seedrate*Fert (Error 1)	6	382.01	63.67	11.05	<.0001
Tillage	1	17.58	17.58	0.11	0.741
Seedrate*Tillage	1	0.06	0.06	0.01	0.919
Fert*Tillage	2	276.90	138.45	24.02	<.0001
Seedrate*Fert*Tillage	2	69.32	34.66	0.22	0.802
Rep*Seedrate*Fert*Tillage (Error 2)	15	2319.51	154.63	26.83	<.0001
day	3	195.63	65.21	11.31	<.0001
Seedrate*day	3	61.84	20.61	3.58	0.017
Fert*day	6	42.73	7.12	1.24	0.295
Tillage*day	3	37.91	12.64	2.19	0.094
Seedrate*Fert*Tillage*day	21	43.07	2.05	0.36	0.996

Height, 2000. (R2= .934 CV=11.92)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	1.96	0.65	8.16	0.000
Seedrate	1	0.04	0.04	0.04	0.856
Fert	2	0.29	0.15	0.15	0.867
Rep*Seedrate	3	0.58	0.19	2.40	0.078
Rep*Fert	6	1.38	0.23	2.87	0.017
Rep*Seedrate*Fert (Error 1)	6	5.94	0.99	12.36	<.0001
Tillage	1	9.16	9.16	16.41	0.010
Seedrate*Tillage	1	0.20	0.20	2.50	0.119
Fert*Tillage	2	0.66	0.33	4.10	0.022
Seedrate*Fert*Tillage	1	1.26	1.26	2.25	0.194
Rep*Seedrate*Fert*Tillage (Error 2)	5	2.79	0.56	6.97	<.0001
day	3	8.91	2.97	37.10	<.0001
Seedrate*day	3	0.18	0.06	0.74	0.535
Fert*day	6	0.93	0.16	1.94	0.090
Tillage*day	3	0.57	0.19	2.38	0.080
Seedrate*Fert*Tillage*day	16	1.91	0.12	1.49	0.137

Height, 2001. (R2= .894 CV=4.96)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	1.93	0.64	9.08	<.0001
Seedrate	1	1.36	1.36	2.42	0.171
Fert	2	2.23	1.11	1.98	0.219
Rep*Seedrate	3	3.38	1.13	15.88	<.0001
Rep*Fert	6	6.86	1.14	16.10	<.0001
Rep*Seedrate*Fert (Error 1)	6	3.38	0.56	7.94	<.0001
Tillage	1	0.02	0.02	0.02	0.901
Seedrate*Tillage	1	1.63	1.63	22.99	<.0001
Fert*Tillage	2	1.88	0.94	13.21	<.0001
Seedrate*Fert*Tillage	2	2.95	1.47	1.21	0.325
Rep*Seedrate*Fert*Tillage (Error 2)	15	18.21	1.21	17.10	<.0001
day	3	14.42	4.81	67.72	<.0001
Seedrate*day	3	0.21	0.07	0.99	0.402
Fert*day	6	0.05	0.01	0.12	0.994
Tillage*day	3	0.23	0.08	1.06	0.371
Seedrate*Fert*Tillage*day	21	1.27	0.06	0.86	0.647

Leaf stage, 2000. (R2= .879 CV=19.8)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	11.40	3.80	14.59	<.0001
Seedrate	1	0.60	0.60	1.34	0.291
Fert	2	1.64	0.82	1.83	0.239
Rep*Seedrate	3	0.43	0.14	0.55	0.651
Rep*Fert	6	1.38	0.23	0.88	0.515
Rep*Seedrate*Fert (Error 1)	6	2.69	0.45	1.72	0.134
Tillage	1	8.68	8.68	5.68	0.063
Seedrate*Tillage	1	0.61	0.61	2.36	0.131
Fert*Tillage	2	0.02	0.01	0.04	0.959
Seedrate*Fert*Tillage	1	0.61	0.61	0.40	0.555
Rep*Seedrate*Fert*Tillage (Error 2)	5	7.64	1.53	5.87	0.000
day	3	36.72	12.24	47.00	<.0001
Seedrate*day	3	0.77	0.26	0.98	0.408
Fert*day	6	1.49	0.25	0.95	0.467
Tillage*day	3	2.03	0.68	2.59	0.062
Seedrate*Fert*Tillage*day	17	5.08	0.30	1.15	0.337

Leaf stage, 2001. (R2= .954 CV=8.08)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	8.89	2.96	32.19	<.0001
Seedrate	1	2.17	2.17	3.78	0.100
Fert	2	2.31	1.15	2.01	0.215
Rep*Seedrate	3	3.20	1.07	11.59	<.0001
Rep*Fert	6	8.94	1.49	16.17	<.0001
Rep*Seedrate*Fert (Error 1)	6	3.45	0.57	6.24	<.0001
Tillage	1	0.04	0.04	0.03	0.874
Seedrate*Tillage	1	1.60	1.60	17.40	<.0001
Fert*Tillage	2	2.74	1.37	14.90	<.0001
Seedrate*Fert*Tillage	2	2.70	1.35	0.93	0.415
Rep*Seedrate*Fert*Tillage (Error 2)	15	21.70	1.45	15.71	<.0001
day	3	129.28	43.09	467.93	<.0001
Seedrate*day	3	0.04	0.01	0.13	0.944
Fert*day	6	0.17	0.03	0.31	0.930
Tillage*day	3	0.76	0.25	2.76	0.046
Seedrate*Fert*Tillage*day	21	1.39	0.07	0.72	0.802

Table A10. ANOVA results for prairie junegrass with two seeding rates, three phosphorus treatments and two tillage treatments grown at the University of Manitoba field station in Carman, Manitoba, 2000 and 2001.

Emergence, 2000. (R2= .716 CV=91.7)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	347.44	115.81	9.38	<.0001
Seedrate	1	9.82	9.82	0.53	0.496
Fert	2	28.00	14.00	0.75	0.513
Rep*Seedrate	3	31.22	10.41	0.84	0.475
Rep*Fert	6	105.34	17.56	1.42	0.220
Rep*Seedrate*Fert (Error 1)	6	112.20	18.70	1.52	0.187
Tillage	1	267.26	267.26	14.66	0.002
Seedrate*Tillage	1	7.87	7.87	0.64	0.428
Fert*Tillage	2	97.65	48.82	3.96	0.024
Seedrate*Fert*Tillage	2	72.46	36.23	1.99	0.180
Rep*Seedrate*Fert*Tillage (Error 2)	12	218.74	18.23	1.48	0.156
day	3	30.21	10.07	0.82	0.490
Seedrate*day	3	57.94	19.31	1.56	0.206
Fert*day	6	12.91	2.15	0.17	0.983
Tillage*day	3	43.96	14.65	1.19	0.322
Seedrate*Fert*Tillage*day	21	143.90	6.85	0.56	0.933

Emergence, 2001. (R2= .899 CV=54.5)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	38.40	12.80	2.98	0.040
Seedrate	1	44.76	44.76	2.59	0.158
Fert	2	39.50	19.75	1.14	0.379
Rep*Seedrate	3	118.88	39.63	9.22	<.0001
Rep*Fert	6	32.31	5.38	1.25	0.295
Rep*Seedrate*Fert (Error 1)	6	103.54	17.26	4.02	0.002
Tillage	1	17.30	17.30	0.32	0.587
Seedrate*Tillage	1	0.59	0.59	0.14	0.713
Fert*Tillage	2	177.20	88.60	20.62	<.0001
Seedrate*Fert*Tillage	2	28.66	14.33	0.26	0.774
Rep*Seedrate*Fert*Tillage (Error 2)	9	489.29	54.37	12.65	<.0001
day	3	21.64	7.21	1.68	0.183
Seedrate*day	3	20.34	6.78	1.58	0.206
Fert*day	6	9.34	1.56	0.36	0.900
Tillage*day	3	2.92	0.97	0.23	0.878
Seedrate*Fert*Tillage*day	19	53.49	2.82	0.66	0.844

Height, 2000. (R2= .699 CV=26.9)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	1.15	0.38	2.51	0.067
Seedrate	1	0.08	0.08	0.22	0.655
Fert	2	0.63	0.32	0.84	0.476
Rep*Seedrate	3	0.51	0.17	1.11	0.352
Rep*Fert	6	4.21	0.70	4.58	0.001
Rep*Seedrate*Fert (Error 1)	6	2.26	0.38	2.46	0.034
Tillage	1	0.01	0.01	0.05	0.829
Seedrate*Tillage	1	0.08	0.08	0.50	0.482
Fert*Tillage	2	0.78	0.39	2.57	0.085
Seedrate*Fert*Tillage	2	1.80	0.90	5.09	0.025
Rep*Seedrate*Fert*Tillage (Error 2)	12	2.13	0.18	1.16	0.331
day	3	2.82	0.94	6.15	0.001
Seedrate*day	3	0.32	0.11	0.70	0.556
Fert*day	6	0.42	0.07	0.46	0.834
Tillage*day	3	0.26	0.09	0.57	0.636
Seedrate*Fert*Tillage*day	21	2.31	0.11	0.72	0.797

Height, 2001. (R2= .743 CV=13.7)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	0.11	0.04	0.14	0.937
Seedrate	1	0.67	0.67	1.53	0.262
Fert	2	0.77	0.39	0.89	0.459
Rep*Seedrate	3	1.63	0.54	1.96	0.131
Rep*Fert	6	3.77	0.63	2.27	0.050
Rep*Seedrate*Fert (Error 1)	6	2.61	0.44	1.58	0.172
Tillage	1	0.54	0.54	1.06	0.330
Seedrate*Tillage	1	0.02	0.02	0.08	0.781
Fert*Tillage	2	2.15	1.07	3.89	0.027
Seedrate*Fert*Tillage	2	2.03	1.02	2.00	0.191
Rep*Seedrate*Fert*Tillage (Error 2)	9	4.58	0.51	1.84	0.082
day	3	5.16	1.72	6.23	0.001
Seedrate*day	3	0.98	0.33	1.18	0.326
Fert*day	6	1.23	0.20	0.74	0.618
Tillage*day	3	0.25	0.08	0.31	0.821
Seedrate*Fert*Tillage*day	19	3.41	0.18	0.65	0.849

Leaf stage, 2000. (R2= .842 CV=24.8)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	2.80	0.93	4.78	0.005
Seedrate	1	0.23	0.23	0.59	0.472
Fert	2	0.80	0.40	1.02	0.415
Rep*Seedrate	3	0.22	0.07	0.37	0.772
Rep*Fert	6	5.10	0.85	4.35	0.001
Rep*Seedrate*Fert (Error 1)	6	2.36	0.39	2.01	0.077
Tillage	1	2.45	2.45	6.96	0.022
Seedrate*Tillage	1	0.11	0.11	0.57	0.453
Fert*Tillage	2	0.89	0.44	2.27	0.111
Seedrate*Fert*Tillage	2	1.46	0.73	2.08	0.168
Rep*Seedrate*Fert*Tillage (Error 2)	12	4.22	0.35	1.80	0.067
day	3	33.43	11.14	57.01	<.0001
Seedrate*day	3	0.35	0.12	0.60	0.620
Fert*day	6	0.41	0.07	0.35	0.909
Tillage*day	3	0.41	0.14	0.70	0.557
Seedrate*Fert*Tillage*day	21	4.04	0.19	0.98	0.493

Leaf stage, 2001. (R2= .873 CV=23.4)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	3	1.14	0.38	1.73	0.172
Seedrate	1	2.03	2.03	2.81	0.145
Fert	2	0.37	0.18	0.26	0.783
Rep*Seedrate	3	1.73	0.58	2.61	0.061
Rep*Fert	6	2.67	0.44	2.02	0.079
Rep*Seedrate*Fert (Error 1)	6	4.34	0.72	3.29	0.008
Tillage	1	1.43	1.43	1.36	0.274
Seedrate*Tillage	1	0.15	0.15	0.66	0.419
Fert*Tillage	2	2.33	1.16	5.28	0.008
Seedrate*Fert*Tillage	2	0.24	0.12	0.12	0.892
Rep*Seedrate*Fert*Tillage (Error 2)	9	9.46	1.05	4.78	0.000
day	3	18.76	6.25	28.41	<.0001
Seedrate*day	3	0.48	0.16	0.73	0.541
Fert*day	6	0.43	0.07	0.32	0.921
Tillage*day	3	2.25	0.75	3.40	0.024
Seedrate*Fert*Tillage*day	19	2.92	0.15	0.70	0.805

Table A11. Mean square ratios of between-population vs. within-population variability for morphological characteristics measured in the reference blue grama populations (WY, MN, BR, MB-MS) grown at the University of Manitoba field station in Carman, Manitoba, 2000.

			ANOVA Mean Squares		
Rank	Abbr.	Character	Population (between-)[‡]	Mean Square Error (within-)[§]	Variability Ratio (between:within) [¶]
1	FLL	Flag leaf length	28055.99	574.52	48.83
2	NOSP	No. spikes per culm	2.14	0.08	25.49
3	FIL	First internode length	23497.32	1228.98	19.12
4	FLW	Flag leaf width	1.25	0.09	13.85
5	ACH	Average culm height	488.99	42.93	11.39
6	RCA	Reproductive culm angle	15.38	1.55	9.92
7	HTC	Height of tallest culm	461.28	63.97	7.21
8	DTF	Days to flowering	506.43	109.38	4.63
9	ISL	Inter-spike length	160.43	35.02	4.58
10	SPWT	Spike weight (5 culms)[†]	0.07	0.02	3.48
11	LSP2	Length of spike 2	110.13	32.81	3.36
12	LSP1	Length of spike 1	86.24	29.83	2.89

[†]Weight of all spikes from a 5-culm random sample taken from each plant.
[‡]Represents the variation associated with between-population distinctness.
[§]Represents the variation associated with within-population distinctness.
[¶]Calculated as X/Y, where X represents the population mean square and Y represents the mean square of the residual error term.

Table A12. Mean square ratios of between-population vs. within-population variability for morphological characteristics measured in the four blue grama populations (WY, MN, BR, MB-MS) grown at the University of Manitoba field station in Carman, Manitoba, 2001.

			ANOVA – Mean Squares		
Rank	Abbr.	Character	Population (between-)[‡]	Error (within-)[§]	Variability Ratio (between : within)[¶]
1	DTF	Days to flowering	2732.98	29.99	91.12
2	PBP	Plant biomass production	141968.39	1767.17	80.34
3	FLL	Flag leaf length	19355.97	347.44	55.71
4	ACH	Average culm height	1476.30	53.43	27.63
5	VSP	Visual Seed Production Score	28.95	1.10	26.25
6	SD	Spring Dormancy	16.77	0.64	26.25
7	CD	Crown Diameter	89.53	3.74	23.95
8	HTC	Height of tallest culm	1372.13	76.49	17.94
9	FIL	First internode length	24360.43	1597.83	15.25
10	SY	Seed Yield (entry)[†]	51608.26	4558.52	11.32
11	FLW	Flag leaf width	0.52	0.06	8.18
12	CNW	Culm node width	0.19	0.02	7.85
13	RCA	Reproductive culm angle	11.65	1.70	6.85
14	ISL	Inter-spike length	81.56	44.30	1.84
15	LSP1	Length Spike 1	23.24	26.73	0.87
16	LSP2	Length Spike 2	16.91	27.17	0.62
17	NOSP	No. spikes per culm	0.05	0.07	0.61

[†]Seed was compiled within each replicate before threshing and weighing.
[‡]Represents the variation associated to between-population distinctness.
[§]Represents the variation associated to within-population distinctness.
[¶]Calculated as X/Y, where X represents the population mean square, and Y represents the mean square of the residual error term.

Table A13. ANOVA results for the mean separations of 12 characters measured in 6 blue grama populations grown at the University of Manitoba field station in Carman, Manitoba, 2000.

Days to flowering R^2=.277 CV=9.9

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	1464.78	292.96	2.62	0.023
Population	6	20941.60	3490.27	31.24	<.0001
Rep*population	30	9720.45	324.01	2.90	<.0001

Height of tallest culm R^2=.121 CV=20.2

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	727.19	145.44	2.19	0.054
Population	6	2180.88	363.48	5.47	<.0001
Rep*population	30	4352.51	145.08	2.18	0.000

Average culm height R^2=.160 CV=21.2

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	585.05	117.01	2.79	0.017
Population	6	2411.13	401.86	9.57	<.0001
Rep*population	30	3554.44	118.48	2.82	<.0001

Flag leaf length R^2=.212 CV=27.6

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	1542.04	308.41	0.56	0.728
Population	6	94686.60	15781.10	28.82	<.0001
Rep*population	30	18449.29	614.98	1.12	0.298

Flag leaf width R^2=.274 CV=21.6

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	2.03	0.41	4.60	0.000
Population	6	5.60	0.93	10.59	<.0001
Rep*population	30	16.82	0.56	6.36	<.0001

Reproductive culm angle R^2=.210 CV=27.2

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	128.55	25.71	16.13	<.0001
Population	6	77.41	12.90	8.10	<.0001
Rep*population	30	131.47	4.38	2.75	<.0001

First internode length R^2=.197 CV=18.8

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	18021.49	3604.30	2.97	0.012
Population	6	94131.71	15688.62	12.92	<.0001
Rep*population	30	99984.11	3332.80	2.74	<.0001

Inter-spike length R^2=.126 CV=20.4

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	647.53	129.51	3.63	0.003
Population	6	1664.67	277.45	7.78	<.0001
Rep*population	30	1380.55	46.02	1.29	0.139

Number of spikes per culm R^2=.144 CV=16.3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	0.59	0.12	1.32	0.254
Population	6	7.31	1.22	13.73	<.0001
Rep*population	30	4.08	0.14	1.53	0.036

Length of spike 1 R^2=.100 CV=16.9

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	336.96	67.39	2.15	0.058
Population	6	746.43	124.41	3.97	0.001
Rep*population	30	1131.62	37.72	1.20	0.210

Length of spike 2 R^2=.100 CV=19.5

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	329.12	65.82	1.97	0.082
Population	6	935.48	155.91	4.66	0.000
Rep*population	30	1640.32	54.68	1.63	0.018

Spike weight R^2=.157 CV=34.1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	0.39	0.08	6.65	<.0001
Population	6	0.36	0.06	5.04	<.0001
Rep*population	30	0.67	0.02	1.88	0.003

Table A14. ANOVA results for the mean separations of 17 characters measured in 6 blue grama populations grown at the University of Manitoba field station in Carman, Manitoba, 2001.

Days to flowering R^2=.300 CV=6.7

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	357.63	71.53	2.18	0.055
Population	6	8423.81	1403.97	42.76	<.0001
Rep*population	30	1305.49	43.52	1.33	0.116

Height of tallest culm R^2=.145 CV=14.8

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	1340.04	268.01	3.35	0.005
Population	6	7685.39	1280.90	16.00	<.0001
Rep*population	30	1641.64	54.72	0.68	0.900

Flag leaf length R^2=.286 CV=24.6

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	15247.93	3049.59	8.85	<.0001
Population	6	70599.85	11766.64	34.14	<.0001
Rep*population	30	22042.55	734.75	2.13	0.000

Flag leaf width R^2=.176 CV=15.7

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	3.59	0.72	11.40	<.0001
Population	6	2.75	0.46	7.27	<.0001
Rep*population	30	4.60	0.15	2.43	<.0001

Reproductive culm angle R^2=.181 CV=27.6

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	134.35	26.87	15.44	<.0001
Population	6	36.36	6.06	3.48	0.002
Rep*population	30	104.25	3.47	2.00	0.001

First internode length R^2=.240 CV=15.9

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	87433.11	17486.62	9.54	<.0001
Population	6	225966.93	37661.16	20.55	<.0001
Rep*population	30	146455.15	4881.84	2.66	<.0001

Inter-spike length R^2=.117 CV=17.9

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	384.74	76.95	1.82	0.106
Population	6	2469.96	411.66	9.74	<.0001
Rep*population	30	1669.70	55.66	1.32	0.121

Number of spikes per culm R^2=.061 CV=13.7

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	0.21	0.04	0.56	0.733
Population	6	0.30	0.05	0.68	0.665
Rep*population	30	3.31	0.11	1.49	0.046

Length of spike 1 R^2=.174 CV=14.2

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	1729.35	345.87	12.17	<.0001
Population	6	1000.35	166.73	5.87	<.0001
Rep*population	30	1968.36	65.61	2.31	0.000

Visual seed yield score R^2=.168 CV=15.2

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	16.17	3.23	2.61	0.024
Population	6	93.10	15.52	12.52	<.0001
Rep*population	30	64.96	2.17	1.75	0.008

Crown diameter R^2=.284 CV=15.5

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	713.99	142.80	33.78	<.0001
Population	6	286.55	47.76	11.30	<.0001
Rep*population	30	219.68	7.32	1.73	0.009

Plant biomass production R^2=.311 CV=38.5

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	37182.81	7436.56	4.23	0.001
Population	6	487897.51	81316.25	46.24	<.0001
Rep*population	30	69017.56	2300.59	1.31	0.126

Spring dormancy R^2=.230 CV=33.9

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	14.03	2.81	4.40	0.001
Population	6	54.85	9.14	14.33	<.0001
Rep*population	30	81.71	2.72	4.27	<.0001

Culm node width		R^2=.219	CV=12.3		
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	2.14	0.43	17.29	<.0001
Population	6	1.67	0.28	11.23	<.0001
Rep*population	30	1.28	0.04	1.72	0.010
Average culm height		R^2=.173	CV=15.6		
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	683.32	136.66	2.18	0.055
Population	6	8124.47	1354.08	21.58	<.0001
Rep*population	30	1448.93	48.30	0.77	0.809
Length of spike 2		R^2=.120	CV=14.61		
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	1269.83	253.97	8.62	<.0001
Population	6	849.34	141.56	4.80	<.0001
Rep*population	30	1032.55	34.42	1.17	0.247
Seed yield		R^2=.583	CV=18.1		
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	27396.47	5479.29	0.93	0.477
Population	6	220144.04	36690.67	6.21	0.000

Table A15. ANOVA results for the mean separations of the coefficients of variation for the 6 blue grama populations combined across years, grown at the University of Manitoba field station in Carman, Manitoba, 2000 and 2001.

		R^2=.421	CV=12.1		
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Year	1	0.0082	0.0082	14.64	0.0003
Rep	5	0.0037	0.0007	1.32	0.2686
Population	6	0.0141	0.0023	4.19	0.0013
Year*population	6	0.0005	0.0001	0.15	0.9892