SEED PRODUCTION IN *Festuca hallii* (Vasey) Piper: ECOTYPIC VARIATION AND PHYSIOLOGICAL MECHANISMS

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ABSTRACT

Plains rough fescue (*Festuca hallii* (Vasey) Piper)) is an important forage grass species in Western Canada. Seeds of this species are in high demand for forage production and habitat restoration. However, the supply of seeds is extremely limited owing to erratic seed production in time and space. Thus, seed producers require a comprehensive understanding of the factors that influence flowering and seed production in this species. This study elucidated the effects of autumn and spring temperatures and moisture conditions on flowering and seed production in *Festuca hallii*. Furthermore, the present study evaluated the morphological and phenological variation existing among the six ecotypes of *Festuca hallii* from Saskatchewan and Manitoba. Temperature and precipitation data as well as observations on flowering of *Festuca hallii* at the Kernen Prairie near Saskatoon, Saskatchewan between 1986 and 2010 were analyzed to examine the effects of temperature and moisture conditions on flowering and seed production. To assess the morphological and phenological diversity among the six ecotypes of *Festuca hallii*, seedlings were grown in the field and transplanted to the greenhouse in November. Plants not flowering in the greenhouse were vernalized under 5^{o}C- 8 h light for 11 weeks. In another experiment, plants were subjected to temperature regimes of $15/5^{o}$C, $10/0^{o}$C and $5/-5^{o}$C with temperature regime having daylength treatments of 12, 8 and a gradually changing daylength from12 to 8 h. Colder than normal temperatures to normal temperatures and wet to normal summers and autumns favored flowering in the following year. Moreover, warmer than normal to normal temperatures in the spring also favored flowering. However, there was no clear pattern in spring moisture conditions that could distinguish between seed producing and non-seed producing years. The occurrence of freezing events in spring negatively affected seed production. Irrigation in late summer and autumn is recommended for seed production in *Festuca hallii*. Differences in morphological and phenological characteristics were evident among the ecotypes of *Festuca hallii*. Vernalization requirements also varied among ecotypes. Overall, 15/5 to 5/-5^{o}C day/night temperature regimes with photoperiods between 12 and 8 h were effective in inducing flowering. Seed mass was highest under 5/-5^{o}C and 12 h to decreasing daylength. Flowering frequency was highest in the seedlings of MCL ecotype, which were vernalized in the field and again were subjected to the similar vernalization treatment. However, the overall flowering frequencies were higher in both MCR and MCL ecotypes under different artificial vernalization treatments. Because of the variation in growth and vernalization requirements among ecotypes, seed sources should be an important consideration for habitat restoration and forage production.

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LIST OF ABBREVIATIONS

SD	Short-day
LD	Long-day
N	Nitrogen
S	Sulphur
P	Phosphorous
K	Potassium
ATP	Adenosine triphosphate
R	Red
FR	Far red
PGRs	Plant growth regulators
GA	Gibberellins
SAM	Shoot apical meristem
IAA	Indole acetic acid
SPI	Standardized precipitation index
GDD	Growing Degree Days
PCA	Principal Component analysis
ANOVA	Analysis of variance
MCR	Macrorie
PAS	Prince Albert National Park (South-end Meadow)
HG	Hague
MCL	Macklin
TF	Turtleford

RM	Riding Mountain
MAT	Mean annual temperature
MT_AO	Mean temperature from April to October
GSL	Growing season length (days)
GDD5	Growing degree days above 5^{o}C
MAP	Mean annual precipitation
PE	Potential evapo-transpiration
RCBD	Randomized complete block design
GLM	General linear model
TSW	Thousand seed weight

1. INTRODUCTION

Plains rough fescue (*Festuca hallii* (Vasey) Piper*)* is a native, perennial grass species of North America. Existence of Fescue Prairies in Canada is at risk because of agricultural conversion and rapid urbanization (World Wildlife Fund 1988). Most of the remnants of the Fescue Prairie remnants are in small isolated patches, surrounded by either agricultural or hay fields (Grilz and Romo 1995). These small fragments are also unprotected and exposed to random changes in land use (Postma and Van Noordwijk 2005). A thorough understanding of the ecological and biological characteristics of *Festuca hallii* is needed for restoring Fescue Prairies.

Demand for seeds of *Festuca hallii* is increasing for habitat restoration and forage production, however, seed production of this species occurs infrequently. Propagation of plants from seeds is often more economical than vegetative propagation, but the lack of sufficient amounts of seeds remains a challenge (Chapman 1960, Jordon et al. 1988). The interplay between genetic potential and environmental factors controls the transitional time between vegetative and reproductive phase in plants (Sindho and Sasakuma 2002). In the Canadian Prairies, available nutrients and soil moisture are the major limiting factors for crop production (Noorbakhsh et al. 2008). Sexual reproduction declines with increasing water stress because seed number is reduced (Tavili 2008). Thus, water stress during seed development reduces yield and seed quality.

Festuca inconsistently produces seeds, thus seed collection is challenging (Johnston and MacDonald 1967), and this erratic nature of seed production may be related to long-term effects of environmental factors (Stout et al. 1981). Initiation of seed head occurs in the autumn and floral development is completed in the following summer (Johnston and MacDonald 1967). Most flowering in *Festuca hallii* occurs between late-May and early-June and seeds shatter in July and

August. Warm weather and sufficient moisture in early summer are conducive for the growth and flowering of *Festuca* (King et al. 1995). The apical meristems remain vegetative throughout the autumn and winter and the initiation of floral primordial occurs in following spring with increasing daylength and temperature. For most perennial, Festucoid species from temperate regions, vernalization and short-days are required for primary induction followed by a secondary induction that needs moderately high temperature with long days (Heide 1990). Moisture availability in autumn or spring may not be effective in enhancing flowering and seed production.

Transition from vegetative growth to the flowering stage is governed by the interaction between genetic and environmental factors. Environmental conditions necessary for seed production in *Festuca hallii* remain undetermined and the causes of variation in seed production from year to year are not clear. Therefore, the first objective of this study was to examine the environmental conditions required for seed production in of *Festuca hallii*, specifically the impacts of autumn and spring temperatures and precipitation on flowering induction, seed formation, and seed quality. Secondly, the study aimed to assess the morphological and phenological diversity among the ecotypes of *Festuca hallii* from Western Saskatchewan and Manitoba.

The following research questions were addressed:

1. What environmental conditions favor flowering in *Festuca hallii*?
2. Does morphological and physiological diversity exist among ecotypes when grown under the same environmental conditions?

3. What is the optimal vernalizing temperature for vernalization and the required daylength for primary induction in *Festuca hallii*?

Based on the research questions listed above, following hypotheses were formulated:

1. Colder and wet fall conditions followed by warm and wet spring conditions, in comparison to the long-term climate average promote flowering in *Festuca hallii*.
2. Under similar environmental conditions, the morphological and phenological features vary among ecotypes of the grass.

2. LITERATURE REVIEW

2.1 *Festuca hallii* and Fescue Prairies in western Canada

Fescue Prairies in Canada are vulnerable mainly due to agricultural conversion and landscape fragmentation (World Wildlife Fund 1988). Most remnants of Fescue Prairies exist as small isolated patches surrounded by either land used for annual cereals and oilseed crops or hay (Grilz and Romo 1995). Furthermore, these small fragments are often unprotected and exposed to random land use changes (Postma and Noordwijk 2005). Fragmentation and isolation ultimately reduce production of genetically suitable seed lots (May et al. 2003). The concern regarding the conservation and protection of biodiversity in the fragmented regions has markedly increased in recent years (Saunders et al. 1991). Conservation of biodiversity can be possible only by protecting the habitats of sufficient quality and quantity to maximize the probability of population persistence (van Jaarsveld et al. 1998). However, major detrimental effects of habitat fragmentation on plant communities have been observed throughout the world (Hernandez-Stefanoni et al. 2008). With proper management strategies Fescue Prairie can be preserved in protected areas. However, *Festuca hallii* in protected areas has less genetic variation than unprotected areas (Qiu et al. 2007). Therefore, restoring Fescue Prairie with genetically variable seed sets can be improved by considering the unprotected areas as well.

2.2 Morphological and ecological characteristics of *Festuca hallii*

Festuca hallii grasslands are generally located on black Chernozemic soil, fine clay-loam with a pH range of 6.6 to 7.3 (May et al. 2003). It is a densely tufted, weakly rhizomatous, perennial grass. The plant can reach a height of 20-60 cm with a crown of 7-10 cm in diameter.

The grey-green 5- nerved leaf blades are 1-1.5 cm wide and up to 30 cm long (Aiken et al. 1984). Panicles of *Festuca hallii* are 6-15 cm long, open to narrow, and erect. Spikelets contain two fertile florets and the third floret is sterile. The awn-less glumes are of similar length to the first lemma (Aiken et al. 1984, Palvik et al. 1985). Although leaf growth starts in spring, culms start to grow from late May to late June. Biomass production reaches its maximum in July. The optimum flowering time is between late-May and early-June and seeds shatter in July and August (Johnston and MacDonald 1967). Thus, regions with warm summer and greater amount of precipitation are conducive for the growth and flowering of *Festuca hallii (*King et al.1995).

2.3. Ecotypic variation in flowering and seed production

According to Turesson (1922), ecotypes are the "intraspecific product of environmental selection arising as a result of genotypic response to a particular habitat" (Gregor and Watson 1960). Turesson (1922) considered ecotypes as genetically based, having diverse morphological, physiological, and phenological traits (Barbour et al. 1987). Ecotypic variation within a species is a consequence of natural selection (McMillan 1960). Ecotypes evolve in diverse geographic regions over time and proper selection can lead to the development of improved and desirable cultivars in short time (Vogel and Pederson 1993). In 1922, a classic common garden study on ecotypic variation was performed by Jens Clausen, David Keck and William Hiesey in California (Barbour et al. 1987). A 323 km transect extended from near sea level (Stanford University), through the Coast Ranges, California's Central Valley and up to the timberline at 3000 m above sea level. A wide range of species were collected from different locations and also were transplanted to common gardens along the transect. Local adaptations in survival, growth, reproduction, and disease resistance were found in many species. Occurrence of natural variation among native species serves as an important opportunity for further development by selection (Fang et al. 2004). Grasslands serve as an excellent model vegetation to study the contributions of ecotypes to community function (McMillan 1960). Different ecotypes have been identified in many species including *Andropogon* L., *Bouteloua* Lag., *Poa* L., *Sporobolus* R.Br., *Sorghastrum* Nash., *Deschampsia* P. Beauv., *Panicum* L., *Fesctuca* L., *Elymus* L., *Koeleria* Pers. etc. (McMillan 1960, and the references therein). Hulbert (1955) demonstrated variation in plant height, pubescence on different plant parts, flowering time, and colour in cheat grass (*Bromus tectorum L.)* ecotypes. Olmsted (1944) reported the occurrence of genetically different types of *Bouteloua* at different latitudes. In blue grama (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths), populations from southern communities flowered earliest under short day conditions (McMillan 1960). Studies on *Bouteloua* (Olmsted 1944) and *Andropogon* (Larsen 1947) suggested that clones from northern communities need longer daylength whereas southern populations need shorter daylength to produce flowers. In little blue stem (*Schizachyrium scoparium* (Michx.) Nash), earlier flowering types are from more western and northern areas while later flowering types are from eastern and southern areas (Cornelius 1947). McMilllan (1959) demonstrated the occurrence of early maturation in the populations from northern latitudes and higher altitudes in *Andropogon, Bouteloua, Sorghastrum, Sporobolus* and, *Panicum*. McMillan (1960) showed a height gradient in *Bouteloua gracilis* and big bluestem (*Andropogon gerardii* Vitman) in a garden experiment in Texas. Clones from northern parts had the shortest flowering culms; the mid-latitudinal and high altitude populations had medium flowering-culms, whereas the clones from southern part had the longest flowering culms. Nonetheless, fluctuations in moisture conditions play a crucial role in ecotypic variation. It has been found that in general, variable patterns in precipitation during the warm season resulted in late flowering in the population with late maturity (McMillan 1959). Adaptations to local soil conditions can result in behavioral differences in the plant populations, such as selection of early maturing and late maturing clones (McMillan 1959). Ecotypic differences are evident in time of flowering and seed hardness in many legumes and non-leguminous annuals along environmental gradients (Del Pozo et al. 2002 and references therein). For example, populations of California burclover (*Medicago polymorpha* L.) differed in phenology, winter vigour, biomass, and seed production along the aridity gradient. Strong positive correlation was observed between days to first flower and the ratio of mean annual precipitation and potential evapotranspiration.

Exposure to abiotic stresses and competition are two important factors that are involved in ecotypic differentiation (Petru et al. 2006). Ecotypes from unfavorable environment show higher stress tolerance while populations from favorable conditions have higher ability to survive under competitions (Liancourt and Tielborger 2009). Early flowering and small sized plants are more common in xeric climate and late flowering and larger plants occur in Mediterranean environment (Aronson et al. 1992, Petru et al. 2006, Liancourt et al. 2009)

2.4 Environmental factors affecting flowering, seed production and seed quality

Environmental conditions experienced by parent plants during reproduction influences the performance of progeny (Blonder et al. 2007). Even trivial differences in parental or grandparental environment can result in a considerable phenotypic divergence (Andalo et al. 1999, and references therein). Among ecological factors, temperature, daylength, light intensity, soil moisture content, and nutrients play the most significant role in flowering and seed production (Menske 2001). High quality seeds have high level of seed germination and produce uniform, vigorous seedlings under various environmental conditions (Dickson 1980). Typically, changes in plant development due to the aforementioned environmental stimuli directly affect floral induction, or indirectly through changes in source/ sink relationships in various plant tissues (Glover 2007).

Three successive phases are required for the flowering of perennial grasses from temperate areas including:- a) induction or chemical differentiation caused by fulfillment of proper temperature and light; b) initiation, the morphological transformation of an induced growing point from vegetative primordia to reproductive primordia; and c) flower development, the formation of macroscopic flowers (Johnston and MacDonald 1967). Accordingly, a considerable amount of research examined seasonal development of shoot apices in different perennial grass species. In general, apical meristems remain vegetative throughout the autumn and winter, floral primordia are initiated the following spring with increasing day length and temperature (Gardner and Loomis 1953). In most perennial Festucoid species from temperate regions, flowering requires vernalization with short days for the primary induction followed by a secondary induction with moderately high temperature and long days (Ofir and Kigel 2006).

Vernalization is a process that encourage flowering through exposure to low temperature for a certain period. It is a prerequisite for the induction of flowering and seed formation in many temperate grasses (Wang et al. 2003). Two important parameters associated with vernalization are: a) the temporal differentiation between the exposure of plants to prolonged cold temperature during winter and the commencement of flowering in the following spring and summer, and b) renewed vernalization requirement in the successive generations (Jung and Mueller 2009). Vernalization requirements prevent plants from entering the reproductive phase until the risk of cold stress has diminished (Flood and Hallorn 1986). Vegetative phase becomes longer and floral induction is delayed in the absence of vernalization. Even though both low temperature and short days are required to induce flowering in Kentucky bluegrass (*Poa pratensis* L.), growth and development of flowers need long daylength with moderate warm temperatures (Peterson and Loomis 1949).

2.4.1 Effects of temperature on flowering and seed production

For perennial grasses from the temperate regions, critical temperature necessary for primary induction vary greatly among and within species (Heide 1994). Interacting with daylength, temperature plays a key role in regulating flowering time. Thus, plants must experience winter weather conditions before they can respond to the proper daylength to induce flowering (Evans, 1964). Commonly under low temperatures (0-6^{o}C), primary induction can take place both under short day and long day conditions (Heide 1990). Low temperature can fully substitute the requirement of short day condition in many temperate grasses. Flowering induction becomes more dependent on short day condition with increasing temperature until a critical temperature of 12-18^{o}C at which induction does not occur regardless of short day or long-day condition and duration of treatments (Heide 1994). High temperature lengthens the duration of the critical photoperiod for flowering whereas diurnal fluctuations in temperatures promote flowering in temperate grasses (Juntilla 1985).

Development of inflorescence occurs under higher temperatures in temperate grasses with dual induction requirements; short days for primary induction in the autumn followed by a secondary induction that needs moderately high temperatures with long days (Johnston and MacDonald 1967, Ofir and Kigel 2006). However, it varies from species to species (Heide 1994, Ofir and Kigel 2006). The formation of inflorescence starts under short day condition; higher temperatures of 21-24^{o}C can substitute the requirement of long day. For species requiring a transition from short day to long day for the initiation of flowering, critical photoperiod increases with rising temperatures (Heide 1994). Exposure to high temperature in some cases may lead to devernalization when primary induction is incomplete or very marginal (Evans 1964, Heide1988, Heide 1994).

Warm years favor early flowering (Penuelas and Filella 2001, Penuelas et al. 2002, Slayback et al. 2003). Advanced fruit ripening is also common in warm years (Menzel 2003, Penuelas et al. 2002). Conversely, delayed flowering and fruiting has been reported in some late flowering species (Penuelas et al. 2002, Menzel 2003, Slayback et al. 2003). Flowering time decreases with increasing temperature up to 25^{o}C in spring, although culm height, size of panicle and the final number of inflorescence are greater under relatively lower temperatures within 12-15^{o}C (Heide 1994). Rate of development in early blooming species may accelerate under warmer spring condition whereas higher temperatures in summer exceeding the optimal range can delay flowering by inhibiting development of reproductive tissues in late flowering species (Sherry et al. 2007). Reduced flowering has been found in Johnson grass (*Shorghum halepense* Pers.) under 16^{o}C in comparison to plants grown under warmer temperature (Ingel and Rogers 1961). Increasing temperature enhances flowering in gamba grass (*Andropogon gayanus* L.) up to an optimum temperature of 25^{o}C in soybean (*Glycine max* L.) up to an optimum temperature of 24^{o}C (Tompsett 1976). A night temperature between 20-24^{o}C functions best to induce flowering in Cocklebur (*Xanthium pennsylvanicum* Wallr) (Salisbury 1963). Cool night temperature exerts inhibitory effect on flowering in many shortday plants including *Andropogon gayanus* and *Xanthium pennsylvanicum*.

2.4.2 Effect of photoperiods on flowering and seed production

Photoperiodism is the response of plants to daylength that allows plants to adapt to seasonal changes in daylength (Wu et al. 2004). This is considered to be the most important and multifaceted interaction between a plant and its surrounding environment. For example, shortdays in autumn induce bud dormancy and cold hardiness in perennials at higher altitudes (Wu et al. 2004) and longdays induce dormancy in several desert species (Schwabe and Nachmony-Bascombe 1963). Changes in photoperiod with seasons are an important factor controlling flowering time in plants (Jung and Mueller 2009). Photoperiodic signals are perceived by phytochrome, the morphogenetic pigments present in the leaf which causes the synthesis and transport of florigenic signals at the shoot apex in a process called photoperiodic induction (Knott 1934, Sage 1992, Heide 1994).

The classification of plants according to their responses to photoperiod is based on flowering responses to daylength. Shortday (SD) and long-day (LD) plants flower in short-day (SD) and long-day (LD) conditions, respectively. However, some plants are day-neutrals, insensitive to daylength and can produce flowers under any photoperiodic conditions (Taiz and Zeiger 2006). Most temperate grasses require dual daylengths for flowering, SD followed by LD (Heide 1997). *Andropogon gayanus,* Jowar (*Sorghum roxburghii* var. hians (Stapf)), and itch grass (*Rottboellia exaltata* L.) are some of the SD plants with a critical daylength between 12 and 14 hours (Tompsett 1976). Exposure to a period of consecutive two weeks of SD is required for anthesis in *Andropogon gayanus* but longer periods of SD treatments enhance its flowering frequency. Plants may produce a stimulus that accumulates until it reaches the optimal level. The same number of shortdays is more effective on the same leaves than distributed on different leaves of the same plant. In *Rottboellia exaltata* under certain circumstances despite being a SD species, LD can accelerate initiation of flowering (Tompsett 1976). Few temperate grasses such as timothy grass (*Phleum prantense* L.), wood bluegrass (*Poa nemoralis* L.), and ice grass (*Phippsia algida* (Sol.) R. Br.) are obligatory LD plants (Cooper 1958, Heide 1992, Heide 1994). Induction and initiation of flowering in these plants occur both under SD and LD conditions but the formation and development of flowers can occur only under LD conditions (Heide 1994). In general, the initiation of flowering is stimulated by the transition from SD to LD conditions. Requirement of secondary induction depends primarily on the degree of primary induction. Fewer number of LD cycles or a shorter photoperiod can cause secondary induction in plants that have experienced more complete primary induction (Peterson 1961, Heide 1994).

2.4.3 Effects of soil moisture on flowering and seed production

Soil moisture is one of the limiting factors in the semi-arid regions of the Canadian prairies (Noorbakhsh et al. 2008). Water stress during seed development affects seed yield and quality (Clinton et al. 2003). Similarly, sexual reproduction decreases with increasing water stress due to the reduction in seed number and height of seed stalks (Tavili 2008). Thus, adequate soil moisture is of great importance for vegetative and reproductive growth especially at the beginning of the growing season and before the onset of flowering (Warringa et al. 1996). Application of water from late May to late September over several years significantly increased flowering in *Festuca hallii* (Palit et al. 2012). Seed production of *Aristida* sp. was reduced at 7% soil moisture content but both flowering and seed production increased at 10% moisture content. Altai wildrye grass (*Leymus angustus* (Trin.) Pilg.) requires soils with high moisture content in August for seed production next year. Therefore, early autumn irrigation is recommended for seed production in dry areas (Saskatchewan Forage Council 2007). Application of water also enhances creeping red fescue (*Festuca rubra* L.) seed production (Fairey et al, 2008). At the successive stages of reproductive development, water stress influences seed yield in ruzi grass (*Brachiaria ruziziensis* (Trin.) Griseb) differently (Wongsuwan 2008). Similarly, soil moisture plays a critical role in regulating the yield and oil content of canola (*Brassica campestris* L.) (Nielsen 1997) and higher amount of precipitation results in the higher seed yield in red clover (*Trifolium pratense* L.) (Steiner and Alderman 2003).

Precipitation patterns during seed production have significant influence on seed germinability in grasses (Herrera-C et al. 2008). Seeds of *Andropogon gerardii* and Indian grass (*Sorgastrum nutans* (L.) Nash) produced in years with above-average precipitation were considerably higher than those years with below average precipitation throughout the growing season (Masters et al. 1993). Strategic irrigation is recommended in windmill grass (*Chloris truncate* (R.) Br.*)* to increase seed quality as insufficient moisture during plant growth and seed development resulted in poor grain filling (Herrera-C et al. 2008). However, heavy rainfall can cause stunted plant growth with low seed germinability and purity. High temperature and relative humidity can also result in reduced pollen survival (Hoglind et al. 2001).

2.4.4. Effect of frost on flowering

Reproductive organs of any plants are the most frost-sensitive parts. Buds, new leaves and other rapidly differentiated organs are more susceptible to frost than the older leaves tissues such as stems or older leaves (Sakai et al. 1981). Flowers and ovaries are often damaged by frost. Nixon and McClain (1969) found that above average temperature in March and April followed by a frost on 10th May can result in severe frost damage in 16 species of trees and shrubs. Similar results were also observed in a long term study by Inouye (2000), who reported that frost affects herbaceous perennials in at least 7 years out of 26 years by damaging buds and open flowers but causes negligible injury to other plant parts such as leaves of stems. By killing the apical meristem, frost causes removal of apical dominance which in turn results in bud mortality (Paige 1992).

2.4.5 Other possible factors that might affect flowering and seed production

Although the predominant controls of flowering and seed production are temperature and photoperiod, both primary and secondary induction can be influenced by a plethora of external and internal factors (Heide 1994).

2.4.5.1 Available Nutrients

Nutrient requirements of plants vary according to growth stages. Among essential nutrient elements, nitrogen (N) is one of the most important elements for seed production and plant growth. Nitrogen fertilizers significantly enhance yield, seed protein concentration and N uptake of plants (William et al. 2008). Nitrogen also enhances grass seed yield by promoting the growth of vegetative tillers and stimulating the growth of flower heads. However, the effect of nitrogen fertilizers on the emergence of fertile tillers varies with the time of fertilizer application (Evans 1954). Application of nitrogen fertilizers before the commencement of primary induction increases flowering in many temperate grasses that require dual inductions (Calder and Cooper 1961, Aamlid 1993). By increasing the number and vigour of inducible tillers in the autumn, nitrogen indirectly promotes flowering and seed production in these species (Aamlid 1993, Heide 1994). A strong interaction between N and sulphur (S) can influence yield of mustard rapeseed (*Brassica napus* L.) (McGrath and Zhao 1996, Ahmed et al. 2005). These two nutrients play critical roles in plants as they are important constituents of amino acids (Ceccoti 1996). Thus, imbalance of N and S resulted in reduced yield in *Brassica* genotypes (Ahmed et al. 2005). However, phosphorus (P) is also a key constituent of ATP (Adenosine triphosphate) and plays significant role in energy transformation in plants and is important in various aspects of reproduction (Hossain et al. 2007). In tomato (*Lycopersicon esculentum* Mill.), deficiency of P for consecutive 10 days resulted in decreased flower number (Menary and Staden 1976). Furthermore, acute P deficiency can cause delay in flower initiation in subterranean clover (*Trifolium subterraneum*L.)(Rossiter1978).

2.4.5.2 Light intensity

Low light intensities are photoperiodically more active (Heide 1994). In case of LD plants, a high proportion of far-red light is ideal for extension of daylength. Vince-Prune (1975) reported that in many grasses, 2 µmolm^{-2}s^{-1} (400-700 nm) was optimal for extension of 8-10 hour of day light. Far red light of 1.3 µmolm^{-2}s^{-1} was adequate for daylength extension in *Phleum pratense* (Cooper 1958) and about 3.3 µmolm^{-2}s^{-1} was effective in orchard grass (*Dactylis glomerata* L.) and some species of *Lolium* (Cooper 1956). Seeds matured under white light with a high red/far red ratio (R/FR) have a higher germination rate in thale cress (*Arabidopsis thaliana* (L.) Heynh.) (McCullough et al.1970).

2.4.5.3 Mowing and removal of aftermath

Removal of aftermath (grass that grows after earlier grass has been cut) and spring cutting influence seed yield in grasses, for example, clipping of aftermath enhances seed yield in Altai ryegrass (Lawrence et al. 1980). Row spacing also influences seed yield in this grass

(Lawrence et al. 1980). Autumn mowing reduces seed yield in the first year seed production leys but it improved seed yield in *Poa Pratensis* in older leys (grassland or pasture) (Aamlid 1993). The removal of tillers in dense and older plants facilitates light conditions for new tillers.

d) Flowering phenology

The timing of flowering is one of the foremost important parameters controlling reproductive success in any plant species. In order to promote seed production, flowering time needs to be in harmony with the surrounding environmental conditions that influence fertilization and seed development (Jung and Mueller 2009). Flowering time also functions as an important determinant of seed yield depending on the local environment and crop production system. Early flowering in cereal crops helps to escape from unfavorable conditions such as extreme heat, drought, frost, and pathogen attack (Poland et al. 2009). Flowering phenology influences seed production as plants with peak flowering time produce seeds with enhanced germination and higher kernel weight (Fang et al. 2004). Daehler (1996) observed that in smooth cord grass (*Spartina alterniflora* Loisel), cross-pollinated flowers produced higher seed set over self-pollinated flowers and seeds from self-pollinated flowers were non-viable. Fang et al. (2004) reported higher seed yield in early flowering populations in *Spartina alterniflora*.

e) Plant Age

Most plants including grasses do not respond to the inductive stimuli and produce flowers before they reach to a certain age or size (Lang 1965). The length of this phase varies considerably in grasses (Cooper 1958). In *Spartina alterniflora*, seed yield was higher in healthy and younger plants (Broome et al. 1974). Vernalization of imbibed seeds at 2^{o}C under dark condition for up to 63 days was highly successful in inducing primary induction in perennial ryegrass (*Lolium perenne* L.) whereas similar treatment for up to 116 days was ineffective in species such as meadow fescue (*Festuca pratensis* Huds.), *Festuca rubra*, orchard grass (*Dactylis glomerata* L.), and *Poa pratensis* (Heide 1994). In case of winter rye (*Secale cereal* L.), even developing caryopsis attached with mother plant can be vernalized by low temperature in autumn (Gregory and Purvis 1938).

f) Carbohydrates

The level of sugar content present in plants plays an important role in floral induction (Bernier 1988). An adequate amount of carbohydrates is a prerequisite for plants to respond to photoperiodic and thermal signals (Heide 1994). The number and rate of growth and development of reproductive tillers increase under higher sugar content (King and Evans 1991). Even when photoperiodic time measurement and induction were not affected by apical sucrose content in poison darnel (*Lolium temulentum* L.), the development of inflorescence can be favored by high sugar availability (King and Evans 1991). Carbohydrate content of the overall plant can also promote the number of flowers in each inflorescence in grapevine (*Vitis venifera* L.) (Lebon et al. 2008). Although the sugar content influences flowering positively in many plants, growth on high sucrose significantly delay flowering time in *Arabidopsis thaliana* (Ohto et al. 2001). However, the extension of late vegetative phase resulted in delayed floral transition.

g) Phytohormones

It has been found that most of the plant growth regulators (PGRs) are linked to the floral transition. As the levels of major PGRs are affected by environmental factors including available nutrients, light quality and available moisture, PGRs such as auxin, cytokinins, and gibberellin can affect flowering time under suitable environmental conditions (Glover 2007). GA (Gibberellin) can substitute LD requirement in many LD plants and long-short day plants when grown under SD conditions (Zeevaart 1985). It can also substitute the vernalization requirements in many cold requiring plants (Lang 1965, Heide 1994). However, GA is usually ineffective or even renders inhibitory effects in most SD plants (Pharis and King 1985). Inhibitors of GA biosynthesis inhibit LD induction, whereas the exogenous application of GA can overcome this problem (Heide 1994). Activities of reproductive shoot apical meristem (SAM) are controlled primarily by cytokinins (Bartrina et al. 2011). While a decrease in cytokinin levels suppressed the activity of SAM in *Arabidopsis thaliana*, an increase of endogenous cytokinins in inflorescence results in the formation of larger inflorescence meristems that are capable to produce more floral primordia (Bartrina et al. 2011). Auxin is another important PGR necessary for the initiation of floral primordial; disruption of auxin biosynthesis in plants leads to the failure of flowering (Cheng et al. 2007). It has been reported that in *Arabidopsis thaliana*, auxin can play two opposite roles in flower development; free indole acetic acid (IAA) produced by different floral organs promotes the development of its own flower but inhibits the development of adjacent flowers (Aloni et al. 2006).

2.4.6 Possible interactions between the environmental factors

Environmental factors, especially temperature and photoperiod are highly interactive in the process of primary induction (Heide 1994). Most plants behave as day neutral plants under low temperatures (0-6^{o}C). With increasing temperature, primary induction becomes more dependent on SD condition until an optimal level is reached; above which primary induction does not take place regardless of photoperiod and duration of exposure.

Interactions between temperature and photoperiod had a pronounced effect on the flowering in wild strawberry (*Fragaria vesca* L.) (Heide et al. 2007). Flowering took place in this species under both 24 and 10 hour photoperiod at 9^{o}C but not at 21^{o}C regardless of photoperiod. Therefore, *Fragaria vesca* behaves as SD, LD or day neutral plants depending on temperature. An interaction between photoperiod and plant age was found in *Andropogon gayanus* (Tompsett 1976). Four weeks of SD induced flowering in older but not in younger seedlings of Asian rice (*Oryza sativa* L.) (Mishra and Khan 1973). Increasing aridity and unpredictable rainfall result in decreasing photoperiod requirements for flower induction (Aronson et al. 1992). In *Medicago polymorpha*, individuals from constant and predictable environments are expected to respond to both temperature and photoperiod (Del Pozo et al. 2002).

2.5 Knowledge Gap

After a prudent review of existing literature, it is evident that the physiological and biochemical regulators of flowering and seed production in perennial grasses have been extensively studied in many plant species. However, a considerable gap in knowledge remains regarding the flowering and seed production in *Festuca halli* such that no specific factor has been found for this species. It is a highly inconsistent seed producer and has a good seed year only in one out of five years. Thus, one can conclude that climate conditions in most years cannot meet the requirements of this species for seed production, which leads to inconsistency in seed production in native prairies. Hence, identifying environmental conditions suitable for seed production of this species has not only practical implications but also scientific value.

3. PATTERNS OF SEED PRODUCTION IN RELATION TO TEMPERATURE AND PRECIPITATION

3.1 Introduction

Plains rough fescue (*Festuca hallii* (Vasey) Piper) is an important perennial grass in western Canada particularly because it provides high quality forage (Willms and Fraser 1992). Grasslands dominated by rough fescue formerly occupied 20% (121,000 km^2) of the grassland region in Saskatchewan; however, urbanization and cultivation have reduced this to only 5-6% of the former area (Grilz and Romo 1995). Reestablishing *Festuca hallii* from seeds is usually more cost effective than vegetative propagation. However, the lack of seeds of *Festuca hallii* remains a major drawback to its use in restorations (Chapman 1960).

Demand for seeds of *Festuca hallii* for habitat restoration and forage production is high. This species is an inconsistent seed producer and a good seed production is not commonin native prairies (J.T. Romo personal coomunication). Similar to most Festucoid perennials from temperate regions, flowering in *Festuca hallii* under natural conditions may require vernalization followed by a secondary induction at moderately high temperatures and long days (Johnston and MacDonald 1967, Ofir and Kigel 2006). Thus, vernalization plays a critical role in flowering induction and seed formation in temperate grasses (Wang et al. 2003). Usually an optimal temperature range exists for floral induction (Fandrich and Mallory-Smith 2006, Heide and Sonsteby 2007).

Soil moisture also a limiting factor in semi-arid grasslands as greater soil moisture or precipitation favors flowering (Craine et al. 2010). Interestingly, greater moisture availability in autumn and spring promote seed production of Altai wildrye grass (*Leymus angustus* Trin.) but autumn or spring moisture individually may not be sufficient in stimulating flowering and seed production (Lawrence et al. 1980). Therefore, temperature and moisture conditions in the autumn may be critical for meeting the vernalization requirements of *Festuca hallii*. However, normal autumn conditions do not seem to be sufficient for inducing flowering in this species since flowering does not occur in most years. On the other hand, environmental conditions in the following spring and summer are also important as regions with warm summer and greater amount of precipitation are conducive for the growth and flowering of *Festuca* species (King et al. 1995).

The objective of this study was to use historical records to evaluate how temperature and soil moisture conditions in the autumn and spring influence flowering in *Festuca hallii*. It was hypothesized that 1) Colder and wet conditions in the autumn, in comparison to the long -term climate average are required to meet the vernalization requirements of *Festuca hallii*, and 2) Normal spring conditions are adequate for flowering but frosts at late stages of flower development are detrimental.

3.2 Materials and methods

3.2.1 Site description

The Kernen Prairie (52°10′N and 106°33′W) of University of Saskatchewan, located 10 km northeast of Saskatoon, was selected as the site to analyze seed production in relation to temperature and precipitation patterns. This site is a 130 ha remnant of fescue prairie surrounded by crop lands (Gross and Romo 2010). It is situated in the Moist Mixed-Grassland Eco-region within the Prairie Eco-zone (Acton et al. 1998) and is classified as Fescue Prairie Association (Coupland 1961). The site is characterized by a mean annual temperature of 2.2°C and mean annual precipitation of 362.6 mm; soil type in this site is predominantly silt and clay loams with a bulk density of 1.14gm/cm^3 (Qiu et al. 2009). The site has not been grazed or hayed since 1967 (Pylypec 1986, Gross and Romo 2010).

3.2.2 Data collection

A 25 year time period (1986-2010) was selected for this study. Noticeable amount of seeds were produced in five years during this period at Kernen Prairie including 1987 (Toynbee 1987), 1996, 2003, 2005 and 2010 (J. T. Romo personal communication (after observing the photographs of Kernen prairie of every spring and summer within the study period)). Temperature and precipitation during 1971-2000 were used as long-term climate normal, which were obtained for the Saskatoon Diefenbaker International Airport Weather Station from Environment Canada (www.weatheroffice.gc.ca). Daily temperature and precipitation data for the study period were obtained for the same station. For years when data were not available or incomplete, other stations within 20 km of the study site were used. Monthly standardized precipitation index (SPI) was calculated as indicator for moisture availability. SPI is a probability index that was developed to give a better representation of abnormal wetness and dryness (Guttman 1999, Table 3.1). SPI uses a gamma or a Pearson Type III distribution and can be created for differing periods of 1-to-36 months. The SPI is a value derived from monthly precipitation that can be effectively used to compare across regions with markedly different climates. The accumulations of Growing Degree Days (GDD) in the springs were calculated after five consecutive days when the average temperature reached 0°C (Fraser 2004).

3.2.3 Data analysis

Monthly average temperatures from July to November of years prior to seed producing years were compared with the long-term climate normal (1970-2000). Categories such as normal or non-normal (colder or warmer) were assigned to each month if the difference between monthly average temperature and the long-term average was smaller or greater than the standard deviations of monthly temperatures, respectively. A similar method was followed for spring temperatures from March to June of seed producing years.

PCA is a bilinear method that represents the main information in the form of a multi-dimensional data table (Kara 2009). Plotting of principal components helps to understand the interrelationships among different variables. Climatic variables such as average temperature and SPI of spring, summer and autumn months (March-November) and annual mean temperatures were used in principal component analysis (PCA) in PCORD version 5 (McCune and Grace 2002). The Gabriel plots (biplots) were generated to demonstrate the relations between seed production (represented by seed producing and non-seed producing years) and climatic variables. Two different PCAs were conducted to study the impact of previous years' spring, summer and autumn conditions (March-November), and current years' spring conditions (March-June), respectively. For the first PCA, average (mean) temperature and precipitation (monthly SPI) of previous year's March to November and mean annual temperature prior to seed producing years and non-seed producing years were used as climatic variables between 1986-2010 were used. Similarly for the second PCA, average (mean) temperature and precipitation (SPI) of spring and early summer (March-June), and average spring temperature (average temperature of March-June) of current years of both the seed producing and non-seed producing years were used as climatic variables between 1986-2010 were used.

The number of days with freezing temperatures during 1-50, 50-100, 100-200, 200-300, and 300-500 GDD were calculated for the springs of seed producing years and years prior to and after seed producing years. The analysis of variance (ANOVA) was then performed to determine the difference in the occurrence of freezing events between the seed producing and non-seed producing years in spring. Means were separated using LSD and the significance level was assumed at 5%.

Table 3.1 Standardized precipitation index (SPI) categories obtained from website of Agriculture and Agri-Food Canada.

SPI value	**Category**
2.00	Extremely Wet
1.60 to 1.99	Severely Wet
1.30 to 1.59	Moderately Wet
0.80 to 1.29	Slightly Wet
0.51 to 0.79	Incipiently Wet
-0.50 to 0.50	Near Normal
-0.79 to -0.51	Incipiently Dry
-1.29 to -0.80	Mildly Dry
-1.59 to -1.30	Moderately Dry
-1.99 to -1.60	Severely Dry
-2.00	Extremely Dry

3.3 Results

3.3.1 Effect of temperature and moisture conditions on seed production

Summer and autumn conditions (July to November) of most years prior to seed producing years (1986, 1995, 2002, 2004, and 2009) were characterized by colder to near normal temperature and wet to near normal moisture conditions (Table 3.2). Except for July

2002, summer temperatures (July and August) of these five years were within the range of near normal to colder than normal (average of 1971-2000). Similarly, except for November 2009, which was warmer, autumn temperatures (September- November) were colder to near normal in comparison with the long term average. As evidenced in SPI, summer months (July and August) of years prior to seed producing years had higher precipitation in comparison to the long term climatic average. Except for August 1986 and July 2009 when precipitation was near normal, July and August of all previous years were within the range of incipiently wet to severely wet. In general, September precipitation was higher to near normal and October was wet to near normal except 1986. November precipitation was inconsistent among those years. Moisture conditions of November were near normal in 1986 and 1995, but were drier in 2002, 2004 and 2009.

Table 3.2 Summer and autumn temperature and precipitation (SPI, Standardized Precipitation Index) prior to seed producing years in *Festuca hallii* since 1986 to 2010 at Kernen Prairie compared to climate normal (average of 1971-2000).

Year	July	August	September	October	November
1986	Near Normal T Severely wet (1.78)	Colder T Mildly dry (-0.97)	Colder T Slightly wet (1.10)	Near normal T Incipiently dry (-0.67)	Near normal T and P (0.22)
1995	Colder T Slightly wet (0.87)	Near normal T Moderately wet (1.45)	Near normal T Extremely dry (-2.83)	Near normal T Slightly wet (1.03)	Colder T Near normal P (0.50)
2002	Warmer T Incipiently wet (0.53)	Colder T Slightly wet (-1.24)	Near normal Incipiently wet (0.83)	Colder T Near normal P (0.25)	Near normal Moderately dry (-1.30)
2004	Near normal T Incipiently wet (-0.97)	Colder T Slightly wet (1.20)	Near normal T and P (-0.23)	Near normal T Incipiently wet (0.79)	Near normal T Severely dry (-1.75)
2009	Colder T Near normal P (0.18)	Near normal T Moderately wet (1.58)	Warmer T Near normal P (0.24)	Colder T Moderately wet (1.35)	Warmer T Severely dry (-1.75)

§ Colder=Colder than climate normal; Warmer=Warmer than climate normal;
T= Temperature; P= Precipitation

The majority of seed production years can be separated from non-seed producing years along Axis-1 and Axis-2 of PCA based on previous year's temperature and precipitation conditions (Figure 3.1). Axis-1 explained 21.5 % of the total variance while Axis-2 explained 14.1 % of the total variance. The majority of temperature-related variables exhibited significant negative correlations with Axis-1. For example, the overall average temperature had an eigenvalue of -0.8843. SPI of April (eigenvalue=0.4412) and July (eigenvalue=0.7031) had strong positive correlation with Axis-1, and June and September SPIs were negatively correlated with Axis-2. Therefore, colder temperature and higher precipitation in the previous year favored seed production of *Festuca hallii* in the following year.

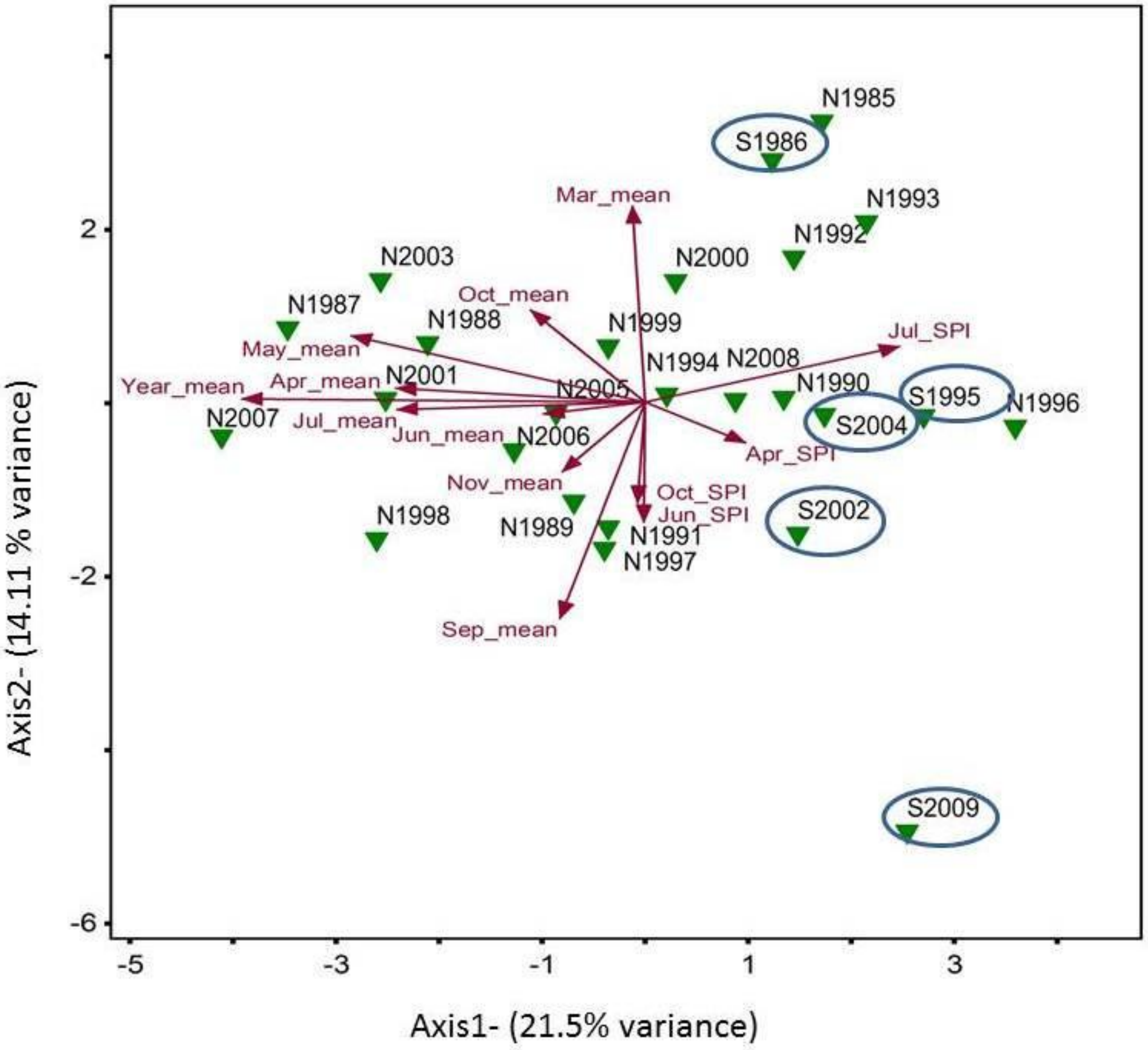

Figure 3.1 Principal component analysis showing the relationship between temperature and moisture conditions of summer and autumn prior to seed production years and seed production of *Festuca hallii* since 1986 to 2010 at Kernen Prairie. Standardized Precipitation Index (monthly) was used to denote the precipitation. Years prior to seed producing years are denoted with “S”; non-seed producing years are denoted with “N”.

3.3.2 Effect of spring weather conditions of seed producing years

Spring months (March-June) in most of the seed producing years within the study period were warmer to near normal (Table 3.3). Out of five seed producing years, March temperatures of 1987, 2003, and 2005 were normal while 2010 March was warmer in comparison to the long term climatic average. April and May temperatures in most of the seed producing years were warmer or near normal. All June temperatures of the five seed producing years were warmer or near normal.

Soil moisture conditions of March 1987, 1996 and 2003 as measured by SPI were normal; March 2005 was slightly wet but March 2010 was severely dry (Table 3.2). April 1996, 2003 and 2010 were from incipiently wet to extremely wet, and April 1987 and 2005 was near normal precipitation. Except for 2003 which was mildly dry, SPIs in May in seed producing years were from near normal to severely wet. June precipitation was inconsistent among seed producing years. Therefore, normal to warmer spring conditions especially during the flowering time (late May to early June) favored seed production *Festuca hallii* even when precipitation was low.

Table 3.3 Spring temperature and precipitation (SPI, Standardized Precipitation Index) of the seed producing years in *Festuca hallii* since 1986 to 2010 at Kernen Prairie compared to climate normal (average of 1971-2000).

Year	**March**	**April**	**May**	**June**
1987	Near normal T and P (0.37)	Warmer T Near normal P (-0.29)	Warmer T Near normal P (-0.09)	Warmer T Mildly dry (-1.11)
1996	Colder T Near normal P (0.01)	Near normal T Incipiently wet (0.69)	Colder T Insipiently wet (0.67)	Warmer T Slightly wet (0.93)
2003	Near normal T and P (-0.46)	Near normal T Moderately wet (1.34)	Warmer T Mildly dry (-0.83)	Near normal T Severely dry (-1.64)
2005	Near normal Slightly wet (1.28)	Near normal T and P (-0.12)	Near normal T and P (-0.28)	Near normal T And Severely wet (1.94)
2010	Warmer T Severely dry (-2.73)	Near normal T Extremely wet (2.16)	Near normal T Severely wet (1.95)	Near normal T Moderately wet (1.43)

§ Colder=Colder than climate normal; Warmer=Warmer than climate normal;
T= Temperature; P= Precipitation

Seed producing years can generally be separated from of non-seed producing years along the Axis-1 based on spring temperature and precipitations, which explain 34.8% of total variance (Figure 3.2). Most spring temperature variables had strong negative correlations with Axis-1. SPIs in spring were correlated with Axis-2 but were not useful in separating seed producing and non-seed producing years, indicating that spring moisture alone is not a limiting factor for seed production.

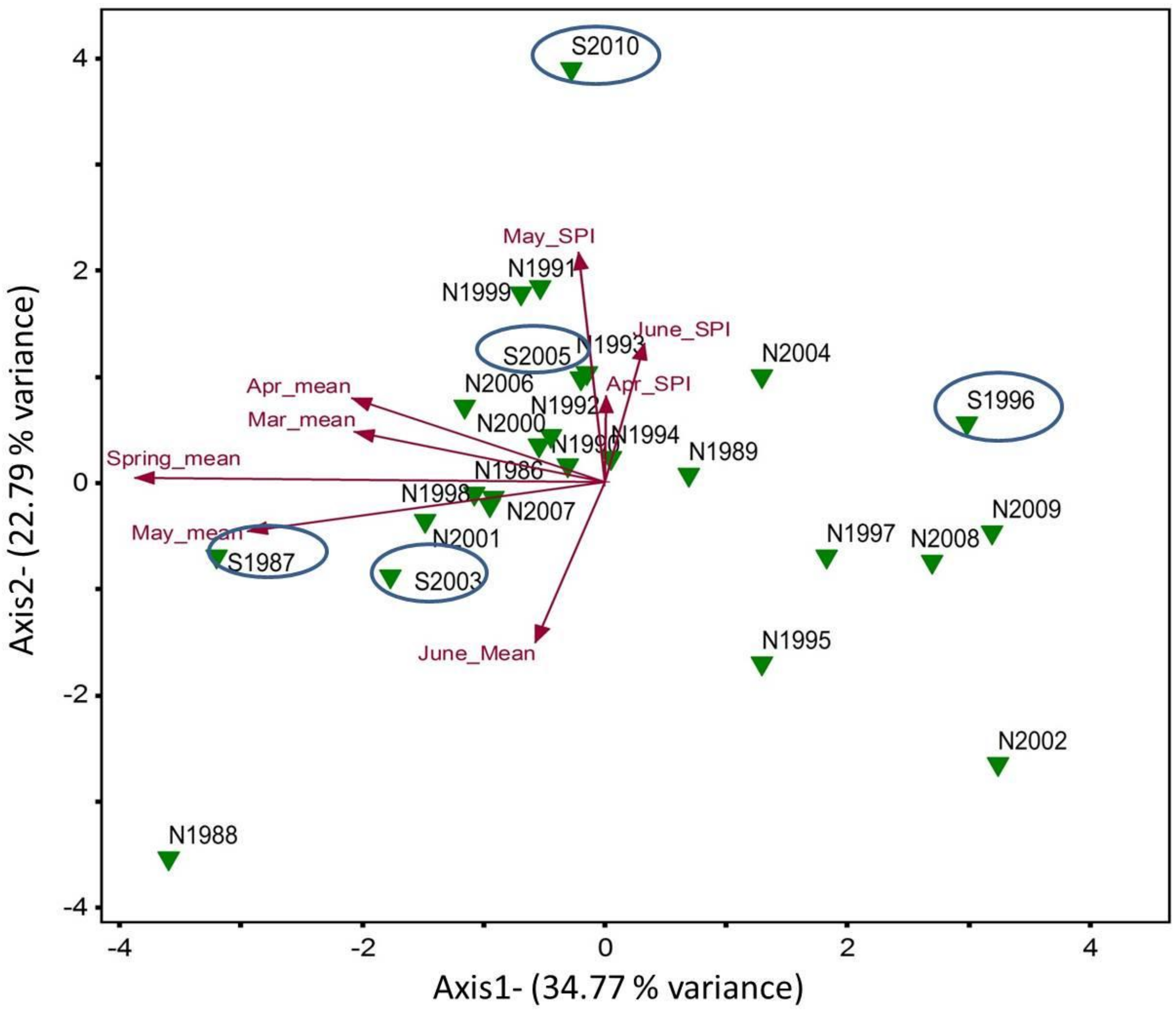

Figure 3.2 Principal component analysis showing the relationship between temperature and moisture conditions of spring of seed producing years and non-seed producing years of *Festuca hallii* since 1986 to 2010 at Kernen Prairie. Standardized Precipitation Index (monthly) was used to denote the precipitation. Years prior to seed producing years are denoted with “S”; non-seed producing years are denoted with “N”.

At the beginning of growing seasons, the number of days with freezing temperature was not different between seed producing and non-seed producing years (Figure 3.3). However, between 50-100 GDD, there was a clear trend of lower smaller number of freezing days in seed producing than non-seed producing years (*P* =0.07). Similar trend was also found between 200-300 GDD. The number of days with freezing temperature after GDD reached 300^{o}C Days was significantly different between seed producing and non-seed producing years (P=0.038), where there were no days with minimum temperature below 0^{o}C in all seed years.

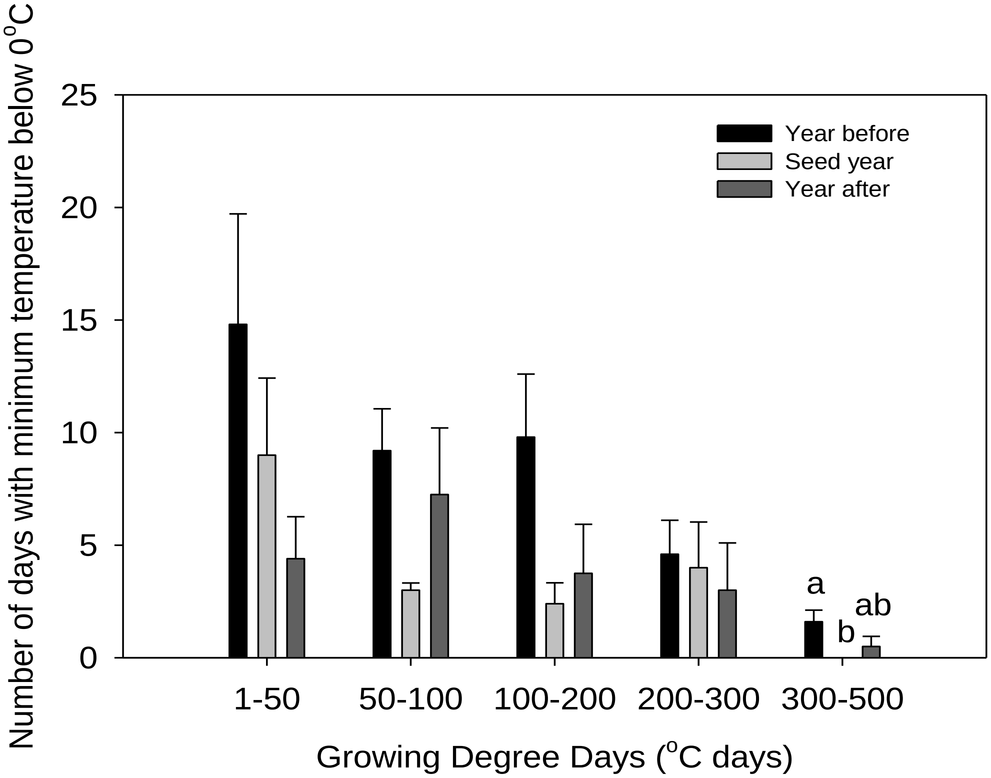

Figure 3.3 Occurrence of freezing events with daily minimum temperature below 0^{o}C in spring (1986-2010) according to periods in Growing Degree Days (GDD) and in seed producing (Seed year) and non-seed producing years (Year before and Year after). Bars are mean ± SE, n=5

3.4 Discussion

Similar to most perennial, temperate grasses, *Festuca* species require low temperatures and short days in autumn and the secondary induction by moderate temperature and long days the following spring and early summer for flowering (Johnston and MacDonald 1967, Aamlid 2005). According to Heide (1994), at least 12 weeks at 6-15^{o}C/short days is necessary for primary induction in red fescue (*Festuca rubra* L.), whereas 16 weeks under same conditions are necessary for meadow fescue (*Festuca pratensis* Huds.). *Festuca hallii* does not produce significant amount of seeds in most years, indicating that normal conditions in autumn and/or spring are not sufficient in meeting flowering requirements. Responses observed in the present study indicate that colder to near normal temperatures in autumn are essential for seed production the following year, confirming a vernalization requirement in Festuca hallii.

Precipitation in the previous summer and autumn favored seed production of *Festuca hallii* in the following year. Seed production of perennial grasses is expected to be higher when environmental conditions in the previous year are favorable for overall plant growth because the state of plants in the previous year potentially influences flowering (Craine et al. 2010). Flowering is a direct function of resource availability, thus flowering is greater in years with higher resource availability (Primack and Hall 1990). Greater seed production in Chinese lyme grass (*Leymus chinesis* Trin.) is attributed to higher autumn precipitation in the previous year and more resource accumulation in meristems (Zhang et al. 2009, Gao et al. 2012). On the contrary, flowering in Tallgrass Prairie flowering can be greater following a dry year (Knapp and Hulbert 1986). Although growth and resource accumulation are limited in dry years, rewetting of soils can lead to pulses of nutrient mineralization that simulate flowering in the following year (Turner and Haygarth 2001, Fierer and Schimel 2002).

Development of inflorescences in perennial grasses occurs in the spring with increasing day length and temperature, but it varies among species (Gardner and Loomis 1953, Heide 1994, Ofir and Kigel 2006). Moderately high temperatures favor seed production in Italian ryegrass (*Lolium multiflorum* Lam.), perennial ryegrass (*Lolium perenne* L.), and *Festuca pratensis* (Akpan and Bean 1976). Responses observed in the present study indicate that average temperatures of most spring months were higher than normal or near the normal in seed producing years of *Festuca haillii*. Cold temperatures during flowering can delay flowering, reduc e seed development and seed yield in tall fescue (*Festuca arundinaceae* Schreb.) (Bean 1971).

Suitable moisture conditions are important for seed production, and it is a key factor driving seed production in *Festuca hallii* (Lamb et al. 2008, Palit et al. 2012). Precipitation and soil water availability are cues to flowering because flowering is strongly associated with resource accumulation (Craine et al. 2010). Thus, inconsistent spring precipitation among seed producing years during in the present study indicates that is not a limiting factor for the flowering of *Festuca hallii*, likely due to the abundance of soil moisture after snow melt in spring. Moreover, previous studies showing enhanced seed production in *Festuca hallii* did not separate the effect of current year precipitation and irrigation in previous years (Palit et al. 2012). Grasses respond differently to precipitation/moisture during flowering. For example, hooded windmill grass (*Chloris cuculata* Bisch.) had a greater seed yield in years with adequate rainfall during flowering (Herrera-C et al. 2008) whereas other perennial grasses including Arizona cotton top (*Digitaria californica* (Benth.) Henr.), sand dropseed (*Sporobolus cryptandrus* Torr.), false Rhodes grass (*Trichloris crinita* (Lag.) Parodi) can produce large amount of seeds even in dry years (Pol et al. 2010).

Reproductive organs of plants are the most frost sensitive parts; buds, flowers and ovaries are often damaged by freezing (Sakai et al. 1981). Nixon and McClain (1969) reported frost damage in 16 species of trees and shrubs in early May in Ohio following above average temperatures in May and April. Frost damaged buds and flowers of herbaceous perennials in at least 7 years out of 26 years (Inouye 2000). For all seed years of the current study, minimum temperatures were not below 0^{o}C after 300 GDD had accumulated. In contrast, non-seed producing years had significantly more freezing events. Freezing events in early spring did not affect flowering while mid-spring freezing events tended to reduce flowering.

In conclusion, colder temperatures especially in the autumn along with higher precipitation in summer and autumn favored seed production the following year for *Fescuta hallii*. Warmer temperatures in spring and early summer favored flowering while precipitation during this period was not essential, likely because of soil water recharge from melting snow. Freezing events during flowering were detrimental, but early spring frosts did not alter seed production. Therefore, timely irrigation in summer and/or autumn is recommended for the next year's seed production of *Festuca hallii*.

4. MORPHOLICAL AND PHYSIOLOGICAL VARIATION AMONG ECOTYPES AND EFFECTS OF AUTUMN TEMPERATURE AND DAYLENGTH ON THE FLOWERING OF *Festuca hallii*

4.1 Introduction

Fescue Prairies in Canada are vulnerable as a major consequence of agricultural conversion (World Wildlife Fund 1988). For the re-establishment of Fescue Prairie throughout the fragmented area, a better understanding of the ecological and biological features of the dominant fescue species is necessary (Romo 2003). The loss of habitats, reduced sizes of patches and greater spatial isolation have slowly led to the loss of biological diversity (Dolt et al. 2005). The genetic variability of native plant species is an important consideration for reclamation (Larson et al. 2000). To maintain the sufficient level of diversity within and among populations a thorough understanding of the ecological variability among the populations is required. Ecotypes evolve in diverse geographic regions over time and proper selection can result in development of improved and desirable cultivars in short time (Vogel and Pederson 1993). Moreover, grasslands serve as an excellent model vegetation to study the contribution of ecotypes to community functions (McMillan 1959). Many grasses including *Andropogon* L., *Bouteloua* Lag., *Poa* L., *Sporobolus* R.Br., *Sorghastrum* Nash., *Deschampsia* P. Beauv., *Panicum* L., *Fesctuca* L., *Elymus* L., *Koeleria* Pers. display ecotypic variation (McMillan 1959, and the references therein). Nonetheless, little information is available about ecotypic variation in plains rough Fescue (*Festuca hallii* (Vasey) Piper), a dominant forage species in Western Canada. Further, variation in morphological and phenological development of ecotypes is largely unexplored.

In most temperate perennial grasses, the regulation of flowering is complex, often with interchangeable effects of temperature and photoperiod (Heide 1994). Floral induction and initiation have been studied in several temperate grasses including annual bluegrass (*Poa annua* L.) (Johnson and White 1997), tall fescue (*Festuca aurundinaceae* Schreb.) (Wang et al. 2003) and jointed goatgrass (*Aegilops cylindrical* Host) (Fandrich and Mallory-Smith 2006). Induction in general can be referred to as the perception, transduction and transmittance of environmental signals resulting in a change in plant development patterns from vegetative to reproductive. The inductive signals are perceived and mediated by a variety of physiological and biochemical processes (Heide 1994). Vernalization is a prerequisite for the induction of flowering and seed formation in many temperate grasses (Wang et al. 2003). There is usually an optimal temperature range for floral induction (Fandrich and Mallory-Smith 2006, Heide and Sonsteby 2007). Thus, critical temperatures and daylengths for primary induction vary among grass species and may also vary among ecotypes from different geographic origins (Heide 1994). The optimum temperature for short day (SD) induction in temperate grasses is approximately 9^{o}C (Heide 1994). In general, the most remarkable primary induction requirement for the duration of vernalization in autumn is found in the genus *Festuca* (Aamlid 2005). According to Heide (1994), at least 12 weeks at 6-15^{o}C/SD is necessary for primary induction in red fescue (*Festuca rubra* L.), whereas 16 weeks under the same conditions is necessary for meadow fescue (*Festuca pratensis* Huds.).

Flowering and seed production in *Festuca* is highly inconsistent (Johnston and MacDonald 1967). Analysis of 25 years weather data revealed that colder temperature in autumn favors next year's flowering in this species (Chapter 3). However, the flowering response of *Festuca hallii* to vernalization has not been studied and ecotypic variation in floral induction and initiation of this species remains unknown. Therefore, the objectives of this study were to determine if six geographically divergent ecotypes exhibit morphological and phenological variation when grown under the same environmental conditions, and also to determine optimal vernalizing temperature and daylength for primary induction in ecotypes of *Festuca hallii*. It was hypothesized that morphological and phenological differences exist among ecotypes, and colder temperature in autumn associated with short daylength favor vernalization procedure in this species.

4.2 Materials and methods

4.2.1 Assessing morphological and physiological variation among ecotypes

4.2.1.1 Plant materials

Fescue seed collections from five sites in Saskatchewan: Macrorie (MCR), Prince Albert National Park (South-end Meadow) (PAS), Hague (HG), Macklin (MCL), Turtleford (TF) and one site in Manitoba- Riding Mountain (RM), were used for this experiment (Figure 4.1). Seeds were collected in August 2005 and stored in paper envelopes in a –20°C freezer at the University of Saskatchewan, Saskatoon. These seed collection sites belong to two Eco-zones (Boreal Forest and Prairie), and four Eco-regions (Aspen Parkland, Boreal Transition, Mid-Boreal Upland and Mixed Grassland) (Table 4.1, Ecological Stratification Working Group 1996). Man annual temperatures of the six sites ranged from 0.5°C to 3.2°C and total annual precipitation averaged322.4 mm to 503.5 mm (Table 4.2).

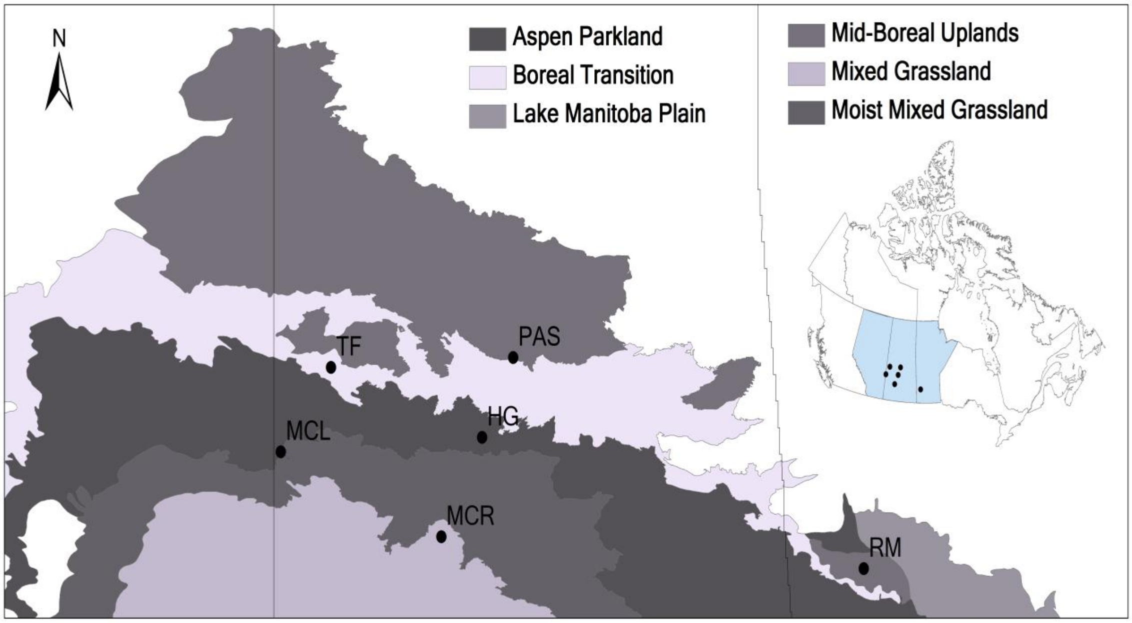

Figure 4.1 Geographic locations of six seed collection sites of *Festuca hallii.*
RM=Riding Mountain, MCR=Macrorie, PAS=Prince Albert National Park (South-end meadow), HG=Hague, MCL=Macklin, TF=Turtleford

Table 4.1 Locations of six seed collection sites of *Festuca hallii* (based on Qiu 2009).

Seed collection	**Eco-zone**	**Eco-region**	**Latitude (N)**	**Longitude (W)**
Riding Mountains (RM)	Boreal Forest	Mid-Boreal Upland	$50^{o}48'35''$	$100^{o}14'42''$
Macrorie (MCR)	Prairie	Mixed Grassland	$51^{o}13'48''$	$107^{o}13'48''$
PA-South-end (PAS)	Boreal Forest	Boreal Transition	$53^{o}35'16''$	$106^{o}02'26''$
Hague (HG)	Prairie	Aspen Parkland	$52^{o}32'22''$	$106^{o}33'17''$
Macklin(MCL)	Prairie	Aspen Parkland	$52^{o}20'56''$	$109^{o}53'02''$
Turtleford (TF)	Boreal Forest	Boreal Transition	$53^{o}27'38''$	$109^{o}03'12''$

Table 4.2 Climatic characteristics of six seed collection sites of *Festuca hallii*. MAT=mean annual temperature, MT_AO=mean temperature from April to October, GSL=growing season length in days, GDD5=growing degree days above 5°C, MAP=mean annual precipitation, and PE=potential evapo-transpiration (adapted from Qiu 2009)

Seed collection	**MAT (°C)**	**MT_AO (°C)**	**GSL (day)**	**GDD5 (°C day)**	**MAP (mm)**	**PE (mm)**
Riding Mountains (RM)	1.2	10.9	173	1406.4	503.5	-95.7
Macrorie (MCR)	3.2	12.5	185	1661.1	322.4	-513.8
PA-Southend (PAS)	0.5	10.4	172	1293.7	441	-219.3
Hague (HG)	1.1	11.1	175	1439.3	397.5	-339.0
Macklin (MCL)	2.1	11.4	178	1473.8	407.6	-344.9
Turtleford (TF)	1.2	10.5	174	1298.4	421.6	-251.5

4.2.1.2 Field Study

Field experiments were conducted at the research farm of Agriculture and Agri-Food Canada, Saskatoon Research Centre (52°07′N and 106°38′W). The soil is a dark brown Chernozem with sand, clay and silt contents of 23, 45 and 33%, respectively. Field capacity of the top 5 cm soil is 57% and bulk density is 1.14g/ cm^3 (Qiu 2005).

In the second week of May 2008, seeds were sown in the field using a randomized complete block design (RCBD). Five blocks, each containing six, 1x1 m^2 plots, were separated by 0.5 m buffer-zones. Within each plot, 200 seeds were hand-sown in four rows at 1cm depth. Plots were irrigated regularly, but seedling emergence rate was low. Additional seeds were germinated and seedlings were grown in growth chambers (Sanyo Versatile Environment Chamber MLR-350H, Sanyo Scientific, USA) at 20°C temperature and 12 h light /12 h darkness. Seedlings were transferred to a shade-house in the field after six weeks, and then transplanted to the plots after two weeks.

In November 2008, seeds were germinated in a growth chamber at 20°C and 12 h light/12 h darkness, and seedlings were grown in a greenhouse until planting in the spring of 2009. Seedlings were transplanted to the field in the second week of May in 2009. Survival of these seedlings in the field was almost 80% and no additional seeding or transplanting was required. Other procedures used in 2008 were repeated in 2009.

4.2.1.3 Artificial vernalization treatment

None of seedlings flowered in the field when they were moved to the greenhouse in the second week of November 2008. There was almost no flowering in those seedlings after three months in the greenhouse. Therefore, it was assumed that those seedlings were not vernalized naturally in the field and were then subjected to artificial vernalization in growth chambers (Sanyo Versatile Environment Chamber MLR-350H, Sanyo Scientific, USA). Seedlings were transplanted to pots (2.5 x 2.5 inch^2) filled with potting soil (Sunshine Germination Mix #3). The vernalization protocol described by Wang et al. (2003) was adopted and modified for this study. Seedlings were kept under 18^{o}C-12 h light for three days, then under 12^{o}C -10 h light for another three days. They were then kept under 5^{o}C- 8 h light for 11 weeks. After the low temperature treatment, the 12^{o}C-10 h light and 18^{o}C-12 h light were repeated. The chambers had T-12-VHO fluorescent tubes and frosted 40 watt incandescent bulbs, with an average RH of 50%. Pots were arranged randomly within each growth chamber and watered regularly. After cold treatment, seedlings were transferred to the greenhouse and were fertilized weekly (NPK: 20-20-20). Average temperature in the greenhouse was about 22^{o}C with 60% average RH and 17 h light.

Seedlings from the 2009 growing season were transferred to the greenhouse in the second week of November 2009. A substantial amount of seedlings flowered after three months in the greenhouse. Seedlings that did not flower were subjected to artificial vernalization treatment after following the method described above.

4.2.1.4 Data collection

The following parameters were measured for both 2008 and 2009 seedlings after vernalization treatment:

a) Average height of vegetative tillers.

b) Total number of vegetative tillers.

c) Flowering time: The date when the flowers were observed for the first time. The first day after vernalization treatment was considered as the day one.

d) Anthesis time: The date when anthers were observed for the first time. The first day after vernalization treatment was considered as the day one.

e) Average number of flowering tillers per plant.

f) Average height of flowering tillers per plant.

Plants were harvested after 10 weeks of growing in the greenhouse. The following parameters were measured:

a) Flowering frequency: Percentage of flowering seedlings in each ecotype.

b) Number of seeds: Average number of seeds per reproductive tiller.

c) Seed mass: Weight (g) of 1000 seeds with five replicates

d) Above-ground biomass: Dry weight of the shoot part of each seedling

e) Dry weight (g) of individual tiller: Above-ground biomass/ number of individual tiller per plant

f) Below-ground biomass: Dry weight of root part of each seedling

g) Rhizomes: Diameter of the rhizomes.

4.2.1.5 Data analysis

General linear model (GLM) was used to determine differences in morphological and physiological characteristics among the six ecotypes of *Festuca hallii* after artificial vernalization in 2008 and 2009 field grown seedlings, respectively. Means were separated using LSD and the significance level was assumed at 5%. Regression analysis was done between the climatic variables of ecotype sites and attributes such as the number of flower heads per plant, flowering time, and anthesis time (Table 4.2). To examine the relationship between vegetative growth and reproduction, regression analysis was done between the number of flower heads per plant and the number of vegetative tillers per plant before flowering (after vernalization treatment).

4.2.2 Assessing the effect of autumn temperature and daylength on flowering

4.2.2.1 Plant materials

Seeds of the six ecotypes were germinated in a growth chamber (as described in Section 4.1) at 20^{o}C, 12 h light/12 h darkness and 50% RH. Seedlings were transplanted to pots (3.5 x 3.5 inch^{2}) filled with potting soil (Sunshine Germination mix #3) when the first leaf was 2 cm long. Seedlings were grown and maintained in the greenhouse for about ten months. They were watered regularly and fertilized (NPK: 20-20-20) weekly.

4.2.2.2 Artificial vernalization treatments to simulate autumn temperature and daylength

Seedlings from the greenhouse were moved to the growth cabinets (Sanyo Versatile Environment Chamber MLR-350H, Sanyo Scientific, USA) and were subjected to three temperature regimes, 15/5^{o}C, 10/0^{o}C and 5/-5^{o}C. Each temperature regime had three daylength treatments: 12, 8 and a gradually changing daylength of 12-10-8 h. There were nine factorial treatments with five replicates. Initially all the seedlings were kept under 15/and 12 h daylength for 3 days. Seedlings for both 10/0^{o}C and 5/-5^{o}C treatments were then moved to 10/0^{o}C for 3 days. Seedlings for the 5/-5^{o}C treatments were then moved to the chamber with assigned temperatures. Light in the chambers was supplied with T-12-VHO fluorescent tubes and 40 watt frosted incandescent bulbs with an average RH of 50%. Total treatment duration was 8 weeks. For the changing day length treatments, plants were subjected to the daylength of 12 h for 2 weeks, 10 h for 2 weeks and then 8 h for 4 weeks. The daylengths of all the chambers were set at 12 h. Shorter daylengths were achieved by covering the plants with three layers of black plastic bags. Plants with the 12 h light treatment were covered with transparent plastic bags for the same duration. At the end of the experiment, all the plants were kept under 15/5^{o}C and 12 h daylength for 3 days then moved to the greenhouse. This experiment was repeated once. After seedlings were transferred to the greenhouse and fertilized (NPK: 20-20-20) weekly. The greenhouse was maintained at 22^{o}C with 60% average RH and 17 h light for approximately 4 months.

4.2.2.3 Data collection

The following parameters were measured before harvesting:

a) Average height of vegetative tillers: Height of plants was measured three times; one week after vernalization treatments, during flowering, after they were done with flowering and seed production when seeds were harvested.

b) Total number of vegetative tillers: This was measured three times as described above.

c) Flowering time: The date when the flowers were observed for the first time. The first day after vernalization treatments was considered as day one.

d) Anthesis time: The date when anthers were observed for the first time.

e) Number of flowering tillers per plant.

f) Number of florets per flowering tiller.

g) Average height of flowering tiller of each plant

Parameters measured after harvesting included:

a) Flowering frequency: Percentage of flowering seedlings under each treatment and in each ecotype

b) Seed mass: The weight (g) of 1000 seeds with 2-5 subsamples of 100 seeds each.

4.2.2.4 Data analysis

A mixed General Linear Model (GLM) was used to determine effects of vernalization treatments on flowering and seed production in the six ecotypes of *Festuca hallii*. As the experiment was conducted twice, the two sets of seedlings were analyzed together. Temperature and daylength and ecotype were considered as fixed factors and number of sets of seedlings as a random factor. Flowering and seed production attributes were treated as response variables. Flowering frequency was calculated according to ecotype to determine the effects of vernalization temperature and daylength. Means were separated using LSD and the significance level was assumed at 5%. All analyses for this study were done using in R 2.8.1 (R Development Core Team 2008).

4.3 Results

4.3.1 Morphological and physiological diversity among ecotypes

The percentage of flowering plants varied among six ecotypes of *Festuca hallii* (Figure 4.2). A few seedlings from 2008 flowered in the greenhouse before artificial vernalization treatment and more seedlings flowered after the treatment. More seedlings from 2009 flowered in the greenhouse before the vernalization treatment than from 2008, but no plants flowered after the vernalization treatment. Seedlings of Macklin (MCL) exhibited the highest flowering frequency (38 %) (Figure 4.2). Hague (HG) and Turtleford (TF) seedlings flowered only after artificial vernalization in 2008, but did not flower in 2009. HG had the lowest percentage (4 %) of flowering frequency among all the ecotypes in both the years. The Macrorie (MCR) ecotype did not flower in either 2008 or 2009.

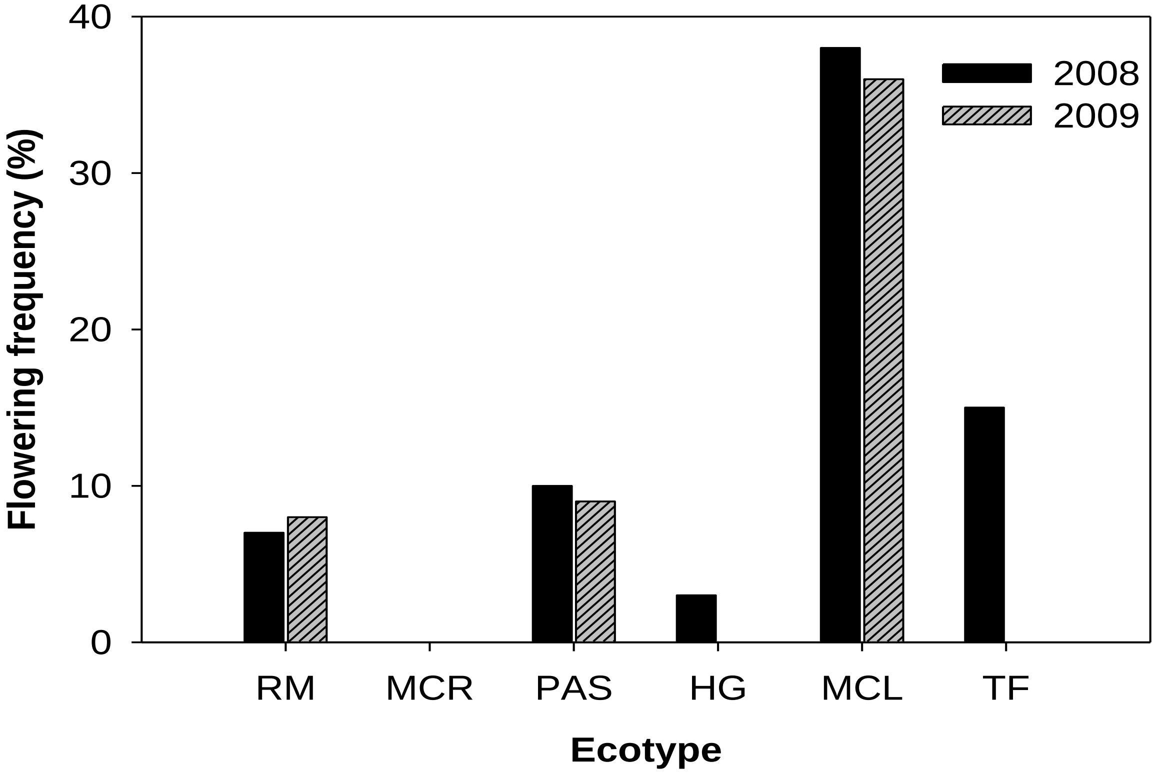

Figure 4.2 Flowering frequency of six Fescue ecotypes in 2008 and 2009. Data for 2008 included flowering plants before and after artificial vernalization treatment; 2009 seedlings did not flower after the vernalization treatment. RM=Riding Mountain, MCR=Macrorie, PAS=Prince Albert National Park (South-end meadow), HG=Hague, MCL=Macklin, TF=Turtleford

Temperatures from late September to October were lower in 2009 than 2008 (Figure 4.3 A). August and September precipitation was higher in 2009 than 2008 (Figure 4.3 B).

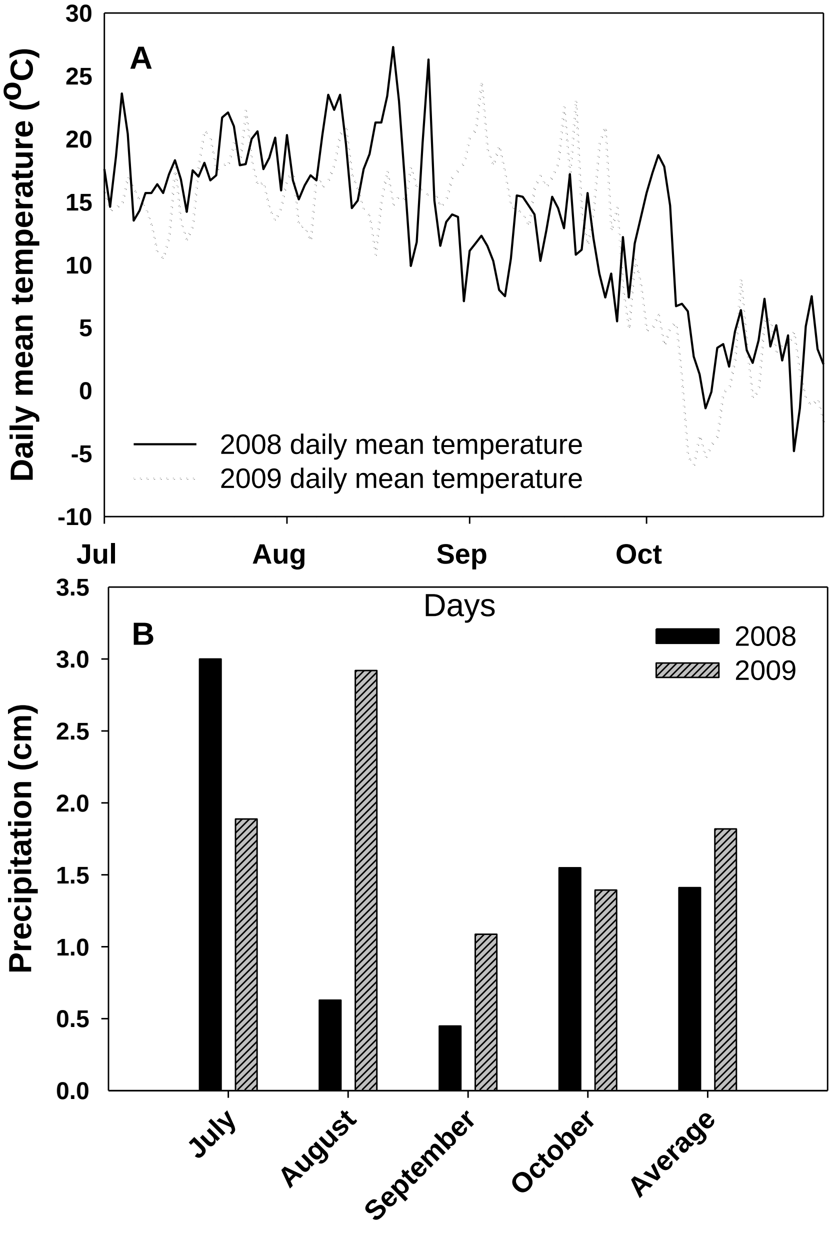

Figure 4.3 Summer and autumn daily average temperature (A) and monthly total precipitation of the study site in Saskatoon in 2008 and 2009. Average=average precipitation of four months (July-October)

Table 4.3 Variation in morphological and reproductive characteristics among six ecotypes of *Festuca hallii* in 2008 after artificial vernalization treatment.

Dependent Variables	**F-value**	***P*-value**
Number of vegetative tillers/plant	9.024	0.000***
Plant height	0.495	0.777
Dry weight of individual tiller (g)	9.725	0.000***
Above-ground biomass (g)	8.567	0.000***
Below-ground biomass (dry weight of root)(g)	3.885	0.008**
Rhizome diameter	12.277	0.000***
Flowering time (days after vernalization treatment)	2.501	0.079
Anthesis time (days after vernalization treatment)	2.65	0.069
Number of flowering tillers	5.000	0.002**
Height of flowering tiller	3.651	0.018*
Number of seeds/tiller	2.232	0.096
Seed mass (Thousand Seed Weight)(g)	12.454	0.000***

*, ** and *** indicate significance at $P \leq 0.05$, 0.01, and 0.001, respectively

There were significant differences in most of the measured vegetative and reproductive attributes among the six ecotypes of *Festuca. hallii* (Table 4.3). After artificial vernalization treatment, the number of vegetative tillers per plant and the dry weight of individual tillers were higher in RM, MCR and PAS ecotypes than the other three (Figure 4.4). The average number of vegetative tillers in these three ecotypes varied between 20 and 30 per plant (Figure 4.4A). However, in HG, MCL and TF ecotypes, the numbers were between 10 and 20 per plant. Ecotypes exhibited consistency in the average plant height (25-30 cm) (Figure 4.4B). Similar to the number of vegetative tillers, aboveground biomass per tiller was also higher in RM, MCR and PAS (0.10 to 0.12 g) than others (Figure 4.4C). The above-ground biomass was higher in MCR ecotype (approximately 2.5 g) than in HG, MCL and TF ecotypes (approximately 1 g) (Figure 4.4D). Belowground biomass was higher in RM, MCR, PAS and MCL than HG and TF ecotypes (Figure 4.4F). Rhizome diameter was highest in PAS (1.8 mm) and lowest in RM (0.5 mm) ecotype (Figure 4.4E).

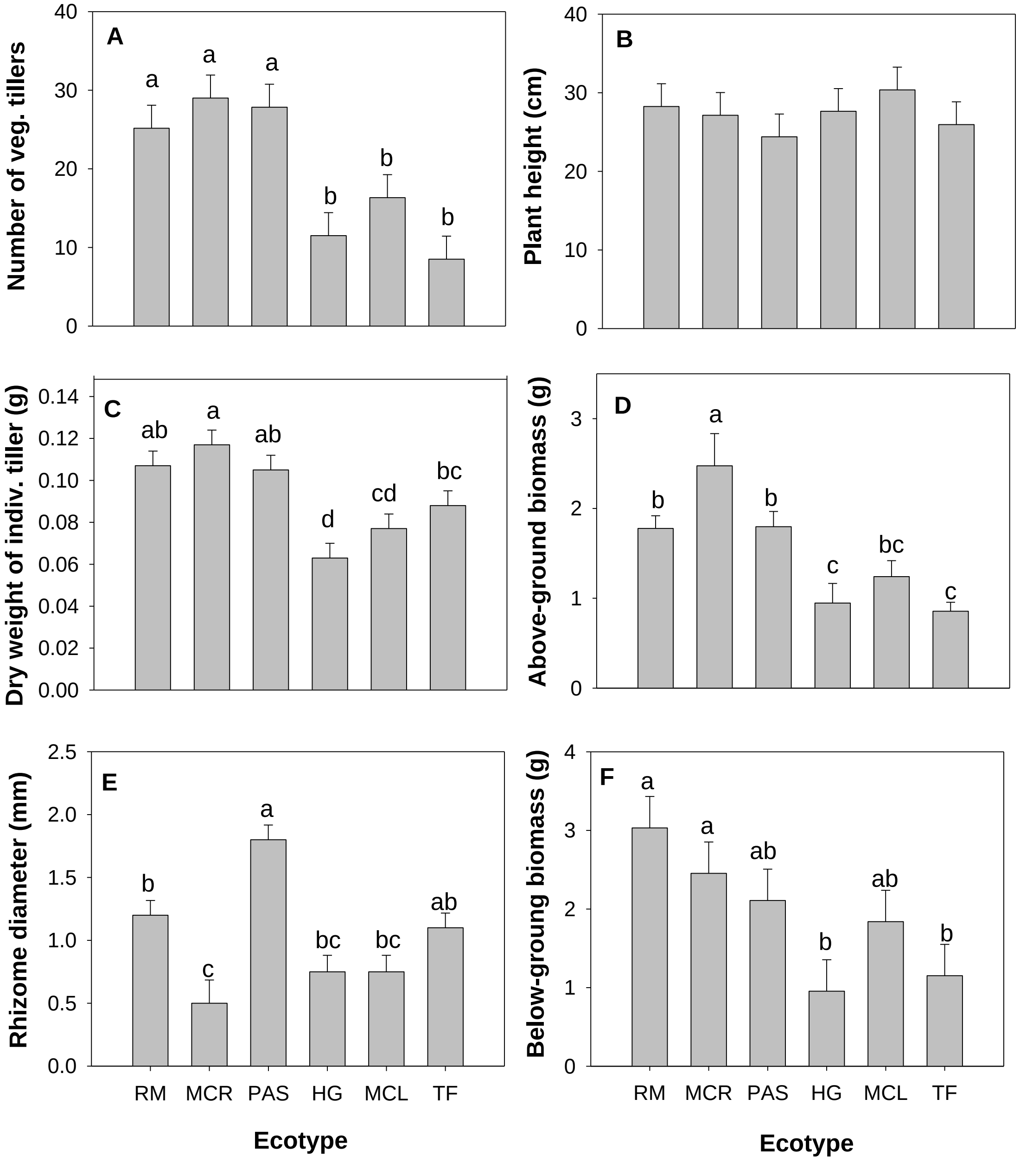

Figure 4.4 Differences in morphological attributes among the six ecotypes of *Festuca hallii* in 2008(*n*=30). Different letters indicate statistically significant difference within each attribute (($P \leq 0.05$).RM=Riding Mountain, MCR=Macrorie, PAS=Prince Albert National Park (South-end meadow), HG=Hague, MCL=Macklin, TF=Turtleford.

The MCL and TF ecotypes required longer time after vernalization treatment (> 20 days) to flower than RM, PAS and HG ecotypes (15-17 days) (Figure 4.5A). RM and MCL ecotypes had longer anthesis time than other ecotypes (Figure 4.5B). The MCL ecotype produced the highest number and the HG ecotype had the lowest number of flowering tillers per plant (Figure 4.5C). The average height of flowering tillers was highest in MCL and TF ecotypes and lowest in the HG ecotype (Figure 4.5D). The Thousand Seed Weight (TSW) was greater than 2 g in the TF ecotype, which was twice of other ecotypes (Figure 4.5 F). Though the average number of seeds/tiller was not significantly different among the ecotypes, it showed a trend (*P*=0.096).

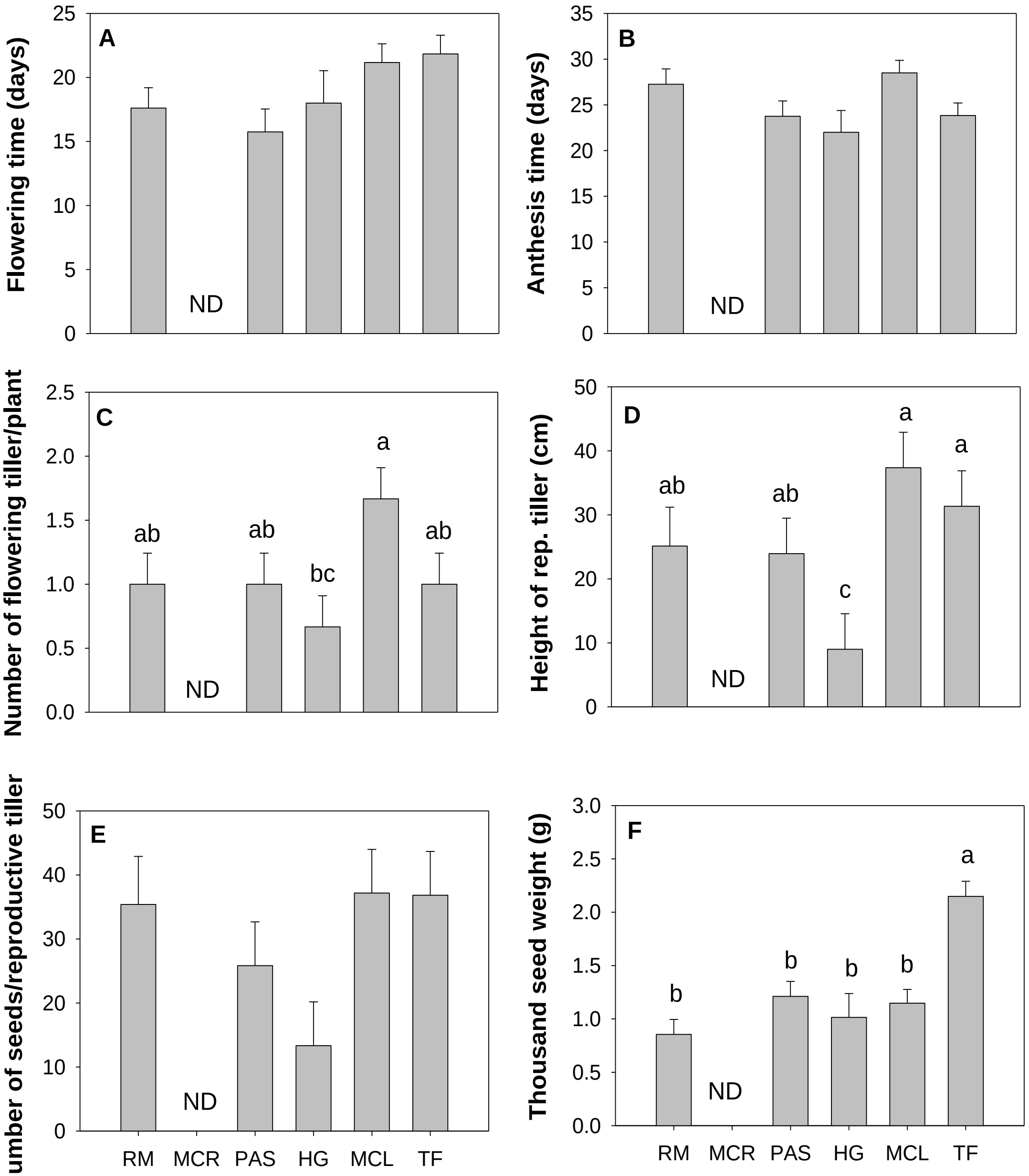

Figure 4.5 Variation in reproductive attributes among six ecotypes of *Festuca hallii* (after 2008, *n*=30). Different letters indicate statistically significant difference within each attribute ((*P*≤0.05). RM=Riding Mountain, MCR=Macrorie, PAS=Prince Albert National Park (South-end meadow), HG=Hague, MCL=Macklin, TF=Turtleford. Seed mass= Thousand Seed Weight. .ND- No data available.

The number of seeds per tiller, flowering time, and anthesis time were not significantly different among ecotypes. The number of flowering tillers per plant was negatively correlated (with the number of vegetative tillers per plant (r = -0.335, P <0.05; Figure 4.6).

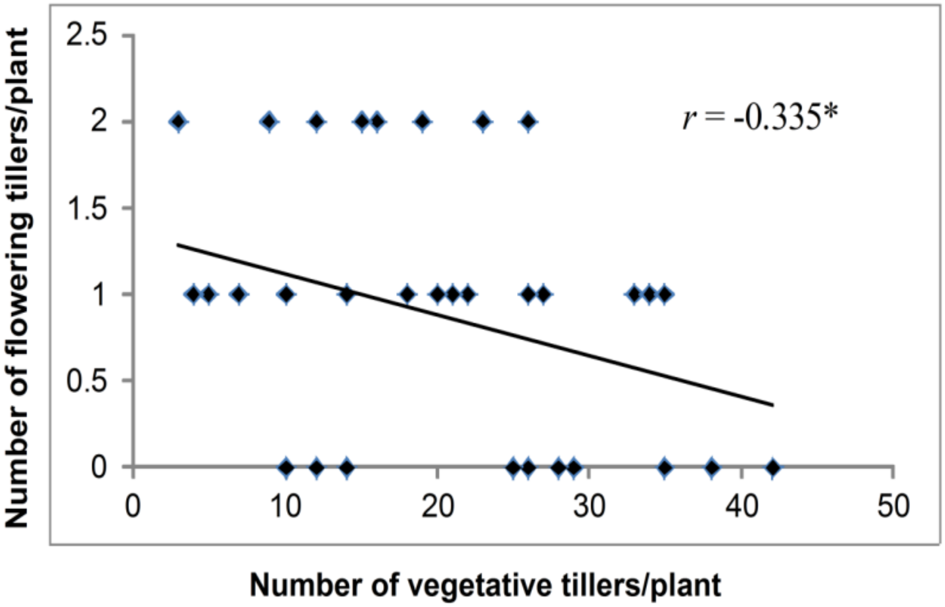

Figure 4.6 Relationship between the number of vegetative tillers/plant and the number of flowering tillers/plant in *Festuca hallii* (2008), n=30, $P \leq 0.05$.

4.3.2 Effects of autumn temperature and daylength on the flowering of Festuca hallii

Most reproductive variables were not significantly affected by treatments including temperature and daylength (Table 4.4). Seed mass (TSW) and floret number/reproductive tiller were the only two attributes significantly affected by these treatments.

Table 4.4 Results of a mixed effect general linear model (considering the number of trials (2) as a random factor) showing the effects of ecotype, temperature and daylength on the induction of flowering and seed production in *Festuca hallii*. *n*=540.

Factors	**Dependent variables**	**F-value**	***P*-value**
Ecotype	Floral heads/plant	3.061	0.080
	Floret number/reproductive tiller	0.802	0.370
	Reproductive tiller (culm) length (cm)	1.608	0.308
	Flowering head length(cm)	1.405	0.481
	Flowering time (number of days)	3.02	0.025*
	Seed mass (Weight of thousand seeds)(g)	8.998	0.003**
Temperature	Floral heads/plant	1.104	0.332
	Floret number/reproductive tiller	4.057	0.018*
	Reproductive tiller (culm) length (cm)	0.26	0.794
	Flowering head length(cm)	0.902	0.526
	Flowering time (number of days)	0.326	0.724
	Seed mass (Weight of thousand seeds)(g)	9.527	0.001**
Day-length	Floral heads/plant	2.728	0.066
	Floret number/reproductive tiller	0.515	0.436
	Reproductive tiller (culm) length (cm)	1.022	0.495
	Flowering head length(cm)	0.467	0.682
	Flowering time (number of days)	2.126	0.136
	Seed mass (Weight of thousand seeds)(g)	17.726	0.000***
Ecotype X Temperature	Floral heads/plant	0.292	0.747
	Floret number/reproductive tiller	0.179	0.835
	Reproductive tiller (culm) length (cm)	0.742	0.677
	Flowering head length(cm)	0.42	0.906
	Flowering time (number of days)	0.907	0.524
	Seed mass (Weight of thousand seeds)(g)	7.815	0.000***
Ecotype X Day-length	Floral heads/plant	1.06	0.347
	Floret number/reproductive tiller	0.608	0.544
	Reproductive tiller (culm) length (cm)	2.352	0.097
	Flowering head length(cm)	2.015	0.142
	Flowering time (number of days)	0.284	0.955
	Seed mass (Weight of thousand seeds)(g)	15.46	0.000***
Temperature X Day-length	Floral heads/plant	0.121	0.975

	Floret number/reproductive tiller	1.572	0.180
	Reproductive tiller (culm) length (cm)	0.619	0.673
	Flowering head length(cm)	0.622	0.671
	Flowering time (number of days)	1.977	0.138
	Seed mass (Weight of thousand seeds)(g)	13.822	0.000***
Ecotype X Temperature X Day-length	Floral heads/plant	1.466	0.211
	Floret number/reproductive tiller	1.04	0.413
	Reproductive tiller (culm) length (cm)	0.809	0.680
	Flowering head length(cm)	1.082	0.365
	Flowering time (number of days)	2.799	0.104
	Seed mass (Weight of thousand seeds)(g)	0.04	0.843

*, **, and *** indicate significance at *P*<0.05, 0.01 and 0.001 respectively

Under different vernalizing temperature and daylength treatments the percentage of flowering plants (flowering frequency) among the six ecotypes varied from 0 to 60% (Figure 4.7). Result of GLM revealed that vernalizing temperature and daylength treatments did not have any significant effect on the flowering frequency. However, significant variation in flowering frequency existed among the ecotypes (*P*=0.007). The highest flowering frequency (60%) was obtained in RM (33.33%) and the lowest in HG (10%).

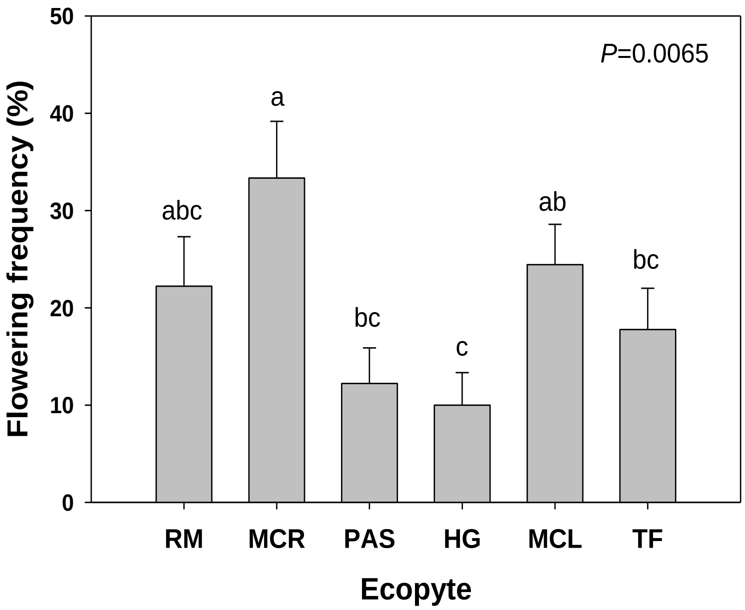

Figure 4.7 Over all percentage of flowering plants in ecotypes of *Festuca hallii* after different vernalizing temperature and daylength treatments. Bars are mean ± SE. (*n*=18). Different letters indicate significant difference ($P \leq 0.05$). RM=Riding Mountain, MCR=Macrorie, PAS=Prince Albert National Park (South-end meadow), HG=Hague, MCL=Macklin, TF=Turtleford.

Ecotype, temperature and daylength did not have statistically significant effects on the number of flowering tillers per plant, although ecotypes (P=0.080) and daylength (P=0.066) trended to affect it (Figure 4.8). Among the six *Festuca hallii* ecotypes, the MCR ecotype tended to produce the highest number of flowering tillers per plant (1.4), while the HG ecotype tended to have the lowest number (0.3). The number of flowering heads per plant was not enhanced by decreasing daylength from 12 to 8 h or decreasing temperature from 15/5°C to 5/-5°C.

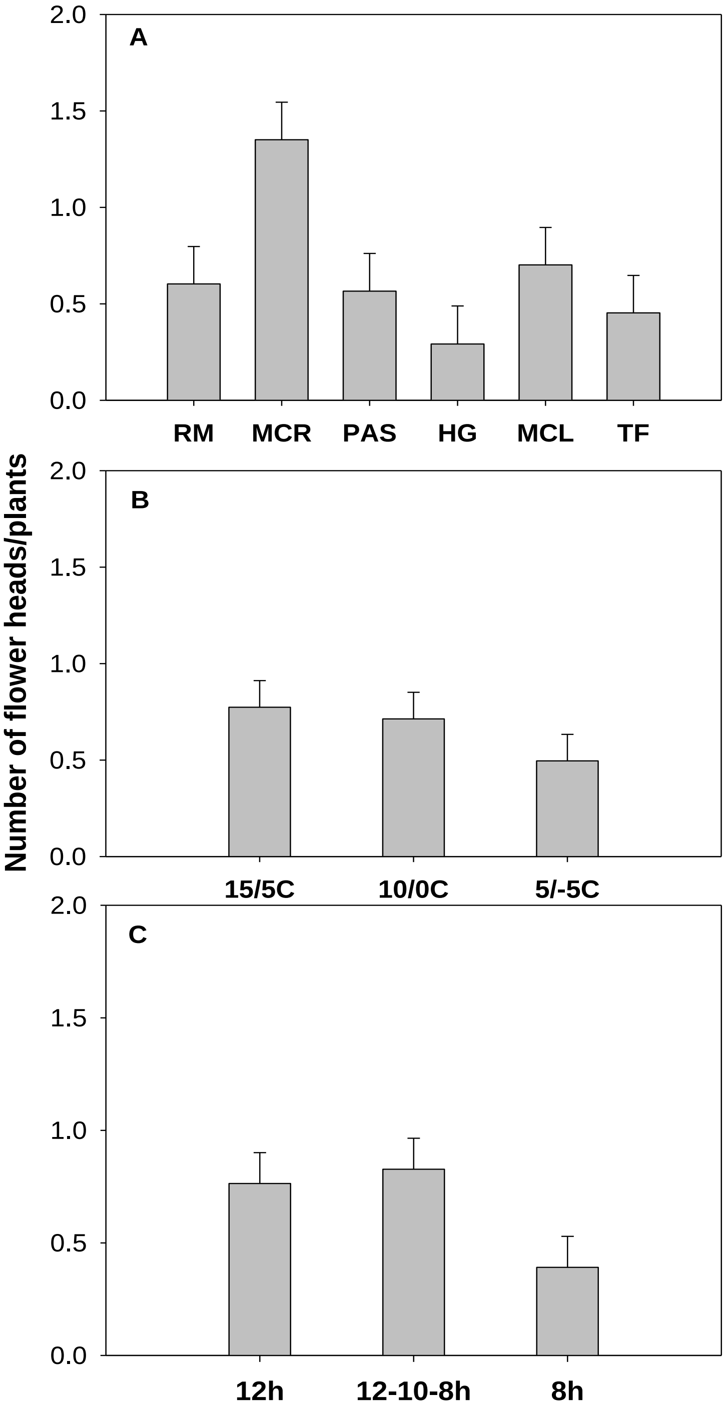

Figure 4.8 Effects of (A) ecotypes, (B) temperatures, and (C) daylengths on the number of flower heads/plant in *Festuca hallii* ecotypes. Bars are mean ± SE. *n*=540.RM=Riding Mountain, MCR=Macrorie, PAS=Prince Albert National Park (South-end meadow), HG=Hague, MCL=Macklin, TF=Turtleford.

Temperature had a significant effect on the number of florets/reproductive tiller, which was higher under15/5^{o}C and 10/0^{o}C day/night temperature regimes than 5/-5^{o}C regime (Figure 4.9). Similar to the number of reproductive tiller per plant, number of florets per reproductive tiller tended to be highest (6) in the MCR ecotype. However, daylength did not significantly affect the number of florets/reproductive tiller.

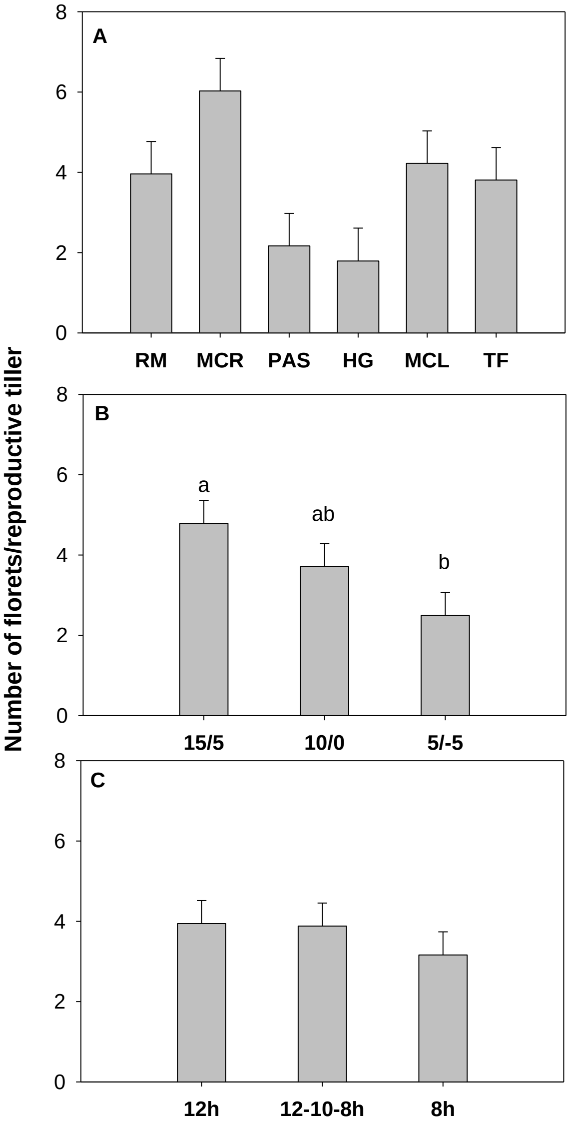

Figure 4.9 Effects of (A) ecotype, (B) temperature, and (C) daylength on the number of florets/reproductive tiller in *Festuca hallii* ecotypes. Bars are mean ± SE. Different letters indicate significant difference within each treatment ($P \leq 0.05$). n=540
RM=Riding Mountain, MCR=Macrorie, PAS=Prince Albert National Park (South-end meadow), HG=Hague, MCL=Macklin, TF=Turtleford

The HG ecotype required significantly longer (*P* =0.025) flowering time after vernalization than other ecotypes (Figure 4.10). Plants vernalized under 15/5^{o}C -12 h tended to have longer flowering time.

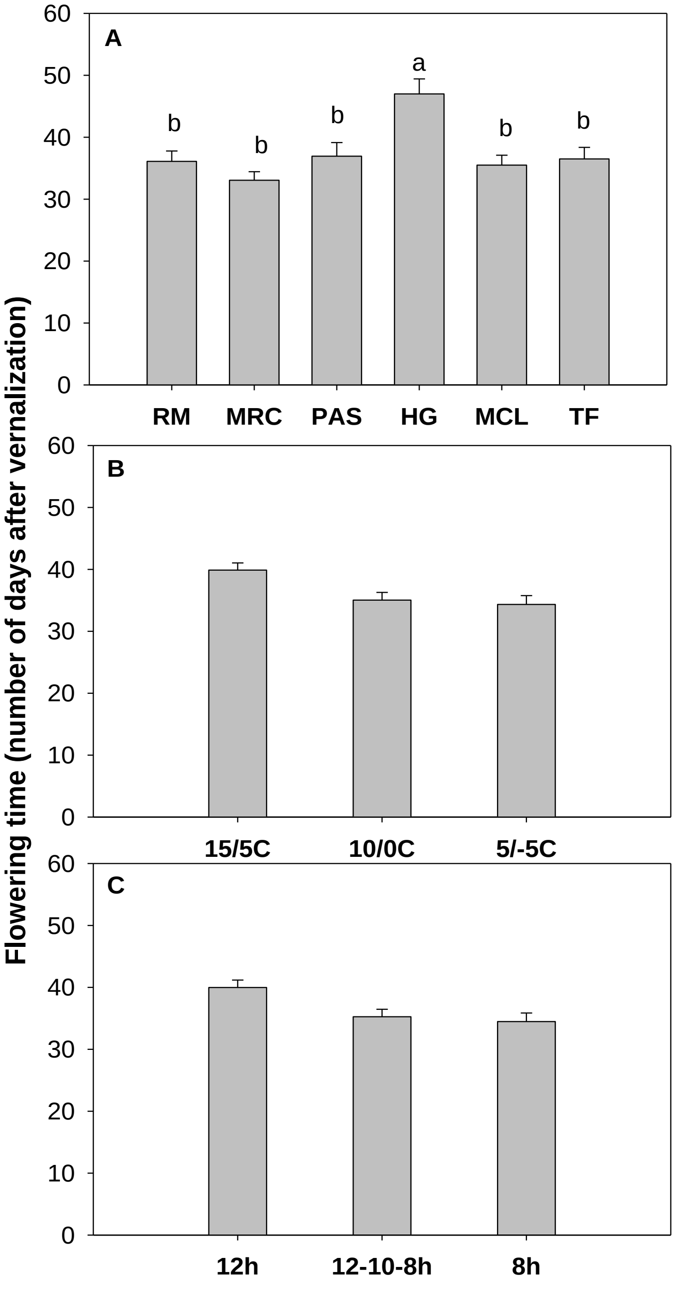

Figure 4.10 Effects of (A) ecotypes, (B) temperatures, and (C) daylengths on the flowering time after vernalization treatment in *Festuca hallii* ecotypes. Bars are mean ± SE. Different letters indicate significant difference within each treatment ($P \leq 0.05$). *n*=540
RM=Riding Mountain, MCR=Macrorie, PAS=Prince Albert National Park (South-end meadow), HG=Hague, MCL=Macklin, TF=Turtleford

Seed mass was significantly affected by the interactions of ecotype, temperature and daylength. In the RM ecotype, seed mass was highest under 15/5^{o}C-8 h vernalization treatment and lowest under 5/-5^{o}C-8 h treatment (Figure 4.11). In the MCR ecotype, plants exposed to 5 /-5^{o}C-12-10-8-h and 5 /-5^{o}C-8 h treatments produced heavier seeds than others. In PAS, HG and TF ecotypes, seed mass were higher under 15/5^{o}C-12 h, 15/5^{o}C-12-10-8 h, and 10/0^{o}C-12 h treatments than others. In the MCL ecotype, 5/-5^{o}C-12-10-8 h and 5/-5^{o}C-8 h treatments resulted in a higher seed mass than others.

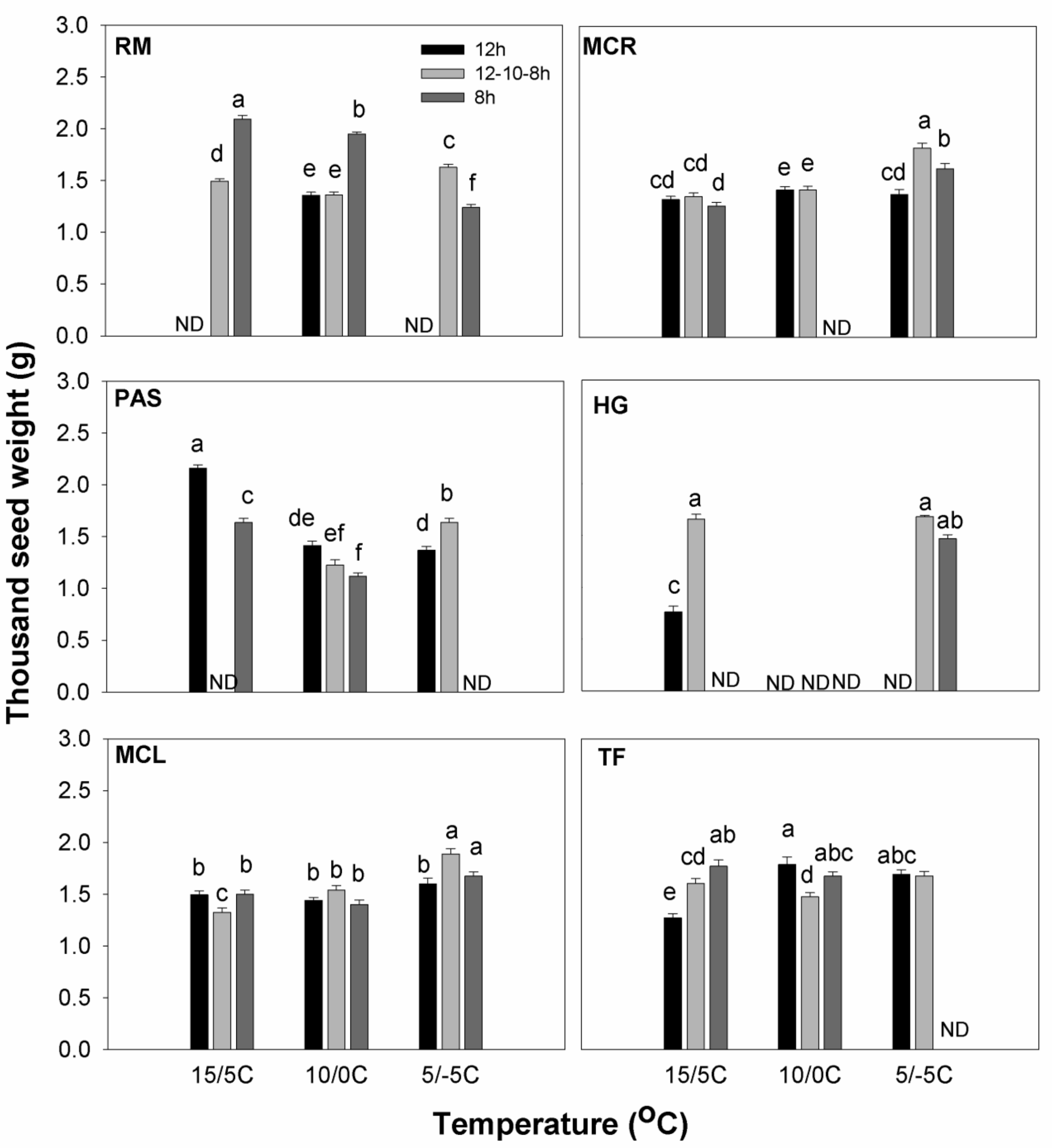

Figure 4.11 Effects of temperature and daylength on seed mass (thousand seed weight (g)) in six ecotypes of *Festuca hallii*, Bars are mean ± SE. (*n*=10).
RM=Riding Mountain, MCR=Macrorie, PAS=Prince Albert National Park (South-end meadow), HG=Hague, MCL=Macklin, TF=Turtleford.
Different letters within an ecotype indicate statistically significant difference ($P \leq 0.05$).
ND= No data available.

4.4 Discussion

The six ecotypes of *Festuca hallii* in our study exhibited differences in most morphological and phenological attributes. The MCR ecotype from the Moist Mixed-grass Prairie Eco-region with warmer and drier climate did not flower when grown in Saskatoon even after artificial vernalization, possibly due to its special requirements in the combination of daylength, temperature and moisture in the autumn. Vegetative attributes of the MCR ecotype, such as average number of vegetative tiller per plant, above-ground and below-ground biomass were greater than, another explanation for the reproductive failure because of possible tradeoffs between vegetative growth and reproductive success in perennial grasses (Lord 1998).

Only a few seedlings transferred from the field in autumn 2008 flowered in the greenhouse before artificial vernalization, but a considerable number of seedlings from 2009 flowered. Therefore, seedlings were successfully vernalized in the field in 2009 but not in 2008. This was validated by observations at the Kernen Prairie where *Festuca hallii* flowered in 2010 but not in 2009 (Chapter 3). The sudden rise in temperature in the autumn of 2008 and relatively low precipitation may have resulted in the failure of meeting the vernalization requirements. On the other hand, the low temperature in autumn of 2009 in combination of relatively high precipitation was adequate to induce flowering in *Festuca hallii*. The analysis of 25 years weather data and observation at the Kernen Prairie indicates that high summer and autumn precipitation and cold temperature favor seed production of *Festuca hallii* in the following year (Chapter 3).

The PAS ecotype from the most northern location tended to have the shortest time to flower. Similar results were reported in little bluestem (*Schizachyrium scoparium* (Michx.) Nash) in which the earlier flowering types were from more western to northern areas and later flowering types from eastern to southern areas (Cornelius 1947). This trend was also found in switch grass (*Panicum virgatum* L.), yellow indiangrass (*Sorghastrum nutans* (L.) Nash.), prairie dropseed (*Sporobolus heterolepsis* Gray.), prairie Junegrass (*Koeleria cristata* (Ledeb.) Schult.), blue grama (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths) (MCMillan 1959). Diverse time to anthesis was found among ecotypes in our study with the shortest anthesis time in the HG ecotype. Difference in anthesis time among ecotypes of *Panicum virgatum* has also been reported (McMillan 1965).

Different vernalization temperature and daylength under controlled conditions did not show any significant effects on flowering frequency and average number of floral heads per plant. However, flowering frequencies were significantly different among ecotypes. The highest flowering frequency (60%) was obtained in RM ecotype under 15/5^{o}C and 12-10-8 h changing daylength. Considerably higher flowering frequencies (near 50%) were obtained in MCR ecotype under 10/0^{o}C-12 h. Interestingly for the MCR ecotype, it did not produce any flowers after natural vernalizing in the field and artificial vernalizing (5^{o}C and 8 h light for 11 weeks), but it achieved the highest flowering frequency (33.33%) when subjected to various levels of vernalizing temperature and daylength treatments within the range of 15/5 to 5/-5^{o}C and 12 to 8 h. The use of alternating temperatures instead of a constant temperature in the second experiment may have contributed this difference. Vernalization temperature significantly influenced the average number of florets per reproductive tiller in *Festuca hallii*. *Festuca* is known to have extreme primary induction requirements (Heide 1994). In three Scandavian cultivars of meadow fescue (*Festuca pretensis* Huds.), 16-20 week at 6^{o}C under 10 h photoperiods were needed for primary induction (Heide 1994). Whereas 9^{o}C was optimal for induction under 8 h daylength, temperatures of 3-6^{o}C were required for induction in continuous light. Temperature and daylength are highly interactive in the primary induction process. Typically in most species, plants become day neutral at low temperatures (0-6°C) and under these conditions primary induction is identical with vernalization process (Lang 1965). In our experiment, the alternating temperatures between 15/5°C and 5/-5°C and 12 to 8 h daylength were not different in inducing flowers, indicating that these conditions are within the range of vernalization requirements of this species.

Seed mass (TSW) varied greatly among ecotypes and at the different levels of vernalizing temperature and daylength. Seed mass was highest in seedlings vernalized under 5/-5°C. Vernalization altered grain weight in spring wheat (*Triticum aestivum* L. cv. Maintou) and there was a decrease in seed mass after vernalization (Iqbal et al. 2011). Seed mass/size has survival values as a large, well developed seed stores more nutrients leading to improved ability of the seedling to survive competition in challenging environments (Steinger et al. 2000, Hovenden et al. 2008). Diverse seed mass among ecotypes after artificial vernalization reflect their adaptation to local environments It is widely acknowledged that vernalization response genes may indirectly contribute to grain yield by influencing flowering time, number of tillers and spikelets (Iqbal et al. 2011). Vernalization affects floral initiation time, leaf number, timing of other growth stages up to emergence of the flag leaf, tiller number and spikelet number (Gororo et al. 2001, Whitechurch and Snape 2003, Iqbal et al. 2011). Positive effect of vernalization on seed yield has been reported in jointed goatgrass (Aegilops cylindrical Host.) and winter wheat (Triticum aestivum L.Norstar.) (Fandrich and Mallory-Smith 2006).

In conclusion, this study demonstrates that ecotypic variation in morphological and reproductive attributes exists in *Festuca hallii* from Western Canada. Vernalization requirements were not met for the ecotype from the Moist Mixed-grass Prairie Eco-region when it was grown in Saskatoon while ecotypes from the other Eco-regions were vernalized in one of the two years in the field (2009). Among the six seed collection sites the average temperature of Macrorie is highest. Therefore the autumn condition of Saskatoon may not be optimal for primary induction in MCR ecotype. The successful vernalization in 2009 and lack of success in 2008 were validated by observations on the flowering of this species in natural grasslands at the Kernen Prairie the following years. This confirms the conclusion from Chapter 3 that cool and wet summer and autumn favors flowering of *Festuca hallii* in the following years. After artificial vernalization treatments, flowering frequency and number of seed heads per plant varied among ecotypes but were not affected by temperature and daylength. Temperatures between 15/5 and 5/-5 ^{o}C, and daylength between 12 and 8 h were within the range of suitable conditions for promoting flowering of *Festuca hallii*, and alternating temperatures were more effective than constant temperatures in meeting the vernalization requirements of this species. Restoration of fescue prairies using native seeds should pay close attention to seed sources because of ecotypic variation in vernalization requirements and growth and development.

5. GENERAL DISCUSSION, CONCLUSIONS AND FUTURE WORK

The present study assessed the drivers of flowering and seed production in plains rough fescue (*Festuca hallii* (Vasey) Piper) from Western Canada by elucidating the impacts of spring temperature and precipitation of spring in seed producing years and summer and autumn temperature and precipitation of the previous years. Furthermore, this study examined the morphological and phenological diversity among the ecotypes of *Festuca hallii* as affected by vernalization treatments.

The analysis of historical weather data (1986-2010) revealed a trend showing that years prior to successful seed years were overall colder (Chapter 3). This is particularly important because it highlights that colder temperatures in late summer and autumn can result in longer vernalization time for successful flower induction and thereby in seed production in *Festuca hallii*. It is widely known that species in the genus *Festuca* have the most extreme primary induction requirements (Aamlid 2005), which may not always be met in the field. This may be the reason that on average, flowering occurred once in five years at the Kernen Prairie. At least 12 and 16 weeks of short day conditions at 6-15^{o}C were required for primary induction in red fescue (*Festuca rubra* L.) and meadow fescue (*Festuca pratensis* Huds.), respectively (Heide 1994). Even though the three temperature regimes, 15/5, 10/0 and 5/-5^{o}C, did not affect the flowering frequency in our experiment, there was a suggestion that 5/-5^{o}C was less effective than higher temperatures. Moreover, the average number of florets/ reproductive tiller was lowest under 5/-5^{o}C temperature regime. Since vernalization is an active process that involves changes in gene expression during cold exposure, vernalization responses only occur at temperatures near or above the freezing point and temperatures below freezing are not effective (Kim et al. 2009).

Results of our study also confirmed the importance of previous years' moisture in reproductive success of this species which is in agreement with a recent study (Palit et al. 2012). The present study further extends the notion that years preceding seed producing years mostly coincided with higher summer and autumn precipitation, thus, highlighting the pivotal role of soil moisture in flowering induction of *Festuca hallii*.

Temperature during the development of the seed head in spring is a critical factor governing seed production in perennial grasses from the temperate region (Ofir and Kigel 2006). This study demonstrates that normal or higher spring temperatures favor successful seed production of *Festuca hallii*. Moderately high temperature enhances seed production in many grass species including Italian ryegrass (*Lolium multiflorum* Lam.), perennial ryegrass (*Lolium perenne* L.), and meadow fescue (*Festuca pratensis* Huds.) (Akpan and Bean 1976). However, the inconsistency in spring precipitation among seed producing years indicates that precipitation in spring is not a limiting factor for flowering of *Festuca hallii* in the Aspen Parkland Ecoregion possibly due to abundant soil moisture from spring snow melt. This study also reveals the importance of the occurrence events at or below 0^{o}C in spring on the flowering of *Festuca hallii*. The onset of these below zero temperatures during flowering was found detrimental, but early spring frosts did not cause any considerable damage to developing floral heads. Consistent colder autumn temperature associated with higher summer and autumn precipitation resulted in successful induction of vernalization in 2009 field-grown seedlings. The successful flowering in seedlings grown in 2009 and unsuccessful flowering in 2008 seedlings were confirmed by observations in the natural grassland at Kernen Prairie the following years. Furthermore, these results support the conclusion that cool and wet summer and autumn favor next year's flowering of *Festuca hallii* (Chapter 3).

Morphological and phenological variation among six ecotypes of *Festuca hallii* reflect their adaptations to local environmental conditions and genetic controls as previously reported in these ecotypes (Qiu 2010). Even under the same environmental conditions, these ecotypes exhibited significant differences in the number of vegetative tillers per plant, above-ground and below-ground biomass, average number of reproductive tillers per plant, average height of reproductive tillers and seed mass. There is usually an optimal temperature range for primary induction (Fandrich and Mallory-Smith 2006, Heide and Sonsteby 2007). Critical temperatures and daylengths for primary induction vary among the grasses and may also vary among the ecotypes from different geographic origins (Heide 1994). In this study, ecotypes of *Festuca halli* had different vernalization requirements, which cannot always be met when planted at locations away from their origins. Vernalization significantly influenced seed mass, which varied greatly among ecotypes and between vernalizing temperature and daylength. Overall, seed mass was highest in seedlings vernalized under 5/-5^{o}C. Seed mass is an important parameter as large and well-developed seeds store more nutrients that help seedlings survive competition in unfavorable conditions (Steinger et al. 2000, Hovenden et al. 2008). The positive effect of vernalization on seed yield has also been reported in jointed goatgrass (*Aegilops cylindrical* Host.) and winter wheat (*Triticum aestivum* L.Norstar.) (Fandrich and Mallory-Smith 2006). Considering the vulnerability of Fescue Prairie and its high seed demand in Canada, this study presents some critical information for seed producers and conservationists. Since variation in growth and vernalization requirements for flowering induction exist among ecotypes, sources of native seeds require considerable attention. For *ex situ* seed production of *Festuca hallii*, vernalization protocols should be modified according to environmental conditions of seed collection sites.

High precipitation and colder temperatures during summer and autumn can be used as indicators for potentially good seed years while irrigation during these periods may also be beneficial.

While the present study reports that moisture at critical stages of reproduction especially in the summer and autumn of previous years positively affects flowering in *Festuca hallii*, the effects of soil moisture during vernalization was not be examined in this study. It is hitherto unexplored how soil moisture plays a critical role by influencing floral induction in *Festuca hallii*. Therefore, future studies should examine the effects of different levels of moisture during vernalization to identify moisture threshold for flowering in *Festuca hallii* ecotypes. Since the length of vernalization affects primary induction process and varies among ecotypes, future studies should investigate the duration of vernalization required to induce flowering in ecotypes of *Festuca hallii*. Furthermore, because vernalization affects seed mass in *Festuca hallii*, molecular tools should be used to unravel the gene expression patterns of vernalization response genes.

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