QUANTIFYING REGROWTH CHARACTERISTICS OF BROMEGRASS

SPECIES (*Bromus*) IN RESPONSE TO DEFOLIATION

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By

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Abstract

Bromegrass species (*Bromus*) can produce high forage yields under the short growing season of western Canada and have excellent nutritive value. Smooth bromegrass (*Bromus inermis* Leyss.) and meadow bromegrass (*Bromus riparius* Rehm.) are the most commonly cultivated bromegrass species. Hybrid bromegrass (*B. riparius X B. inermis*) was developed in Canada by hybridizing smooth and meadow bromegrass. Regrowth potential differs among these three bromegrass species, but the morphological and physiological basis for these differences is unclear. Regrowth characteristics of three bromegrass species following defoliation to 5cm at the vegetative and stem elongation stages of growth were studied in the field and greenhouse. Above-and below-ground dry matter production, leaf area index (LAI) development, individual leaf area expansion, leaf-to-stem ratio, photosynthetic rate, tiller and axillary bud development, etiolated regrowth, and nitrogen concentration in stem bases were evaluated.

Regrowth was similar among the three species when defoliated at the vegetative stage. Meadow bromegrass consistently produced more (P≤0.05) above-and below-ground dry matter than smooth bromegrass following defoliation at the stem elongation stage, while that of hybrid bromegrass was generally intermediate to the other two species. Individual leaf photosynthetic rates did not differ among the three species. Individual leaf area expansion rate was faster (P≤0.05) in smooth bromegrass than meadow and hybrid bromegrass. LAI of the three bromegrass species increased linearly with days of regrowth ($r^2 \geq 0.88$, P≤0.05), and the increase was greatest in meadow bromegrass, intermediate in hybrid bromegrass, and least in smooth bromegrass in all stages of defoliation. Similarly, the leaf-to-stem ratio was highest in meadow bromegrass, intermediate in hybrid bromegrass, and lowest in smooth bromegrass following all defoliations.

Defoliation at the vegetative stage had no effect ($P \geq 0.05$) on tiller development relative to the undefoliated treatment, whereas tiller development was negatively affected by defoliation at the stem elongation stage. After 60 days of regrowth, final tiller density was greatest in meadow bromegrass, intermediate in hybrid bromegrass, and least in smooth bromegrass in the field. A lower proportion of tillers in meadow bromegrass reached the reproductive stage compared to the other two species. The final tiller density following defoliation was similar among species in the greenhouse. Total buds tiller^{-1} and elongated buds tiller^{-1} were similar ($P \geq 0.05$) among three species following defoliation at each growth stage; however, defoliation at stem elongation stage visually delayed bud development. Etiolated regrowth was greater in meadow and hybrid bromegrass ($P \leq 0.05$) than smooth bromegrass 10 days after defoliation, but was similar thereafter. Concentration of N in stem bases was similar among species, but decreased with advancing maturity.

Rapid regrowth of meadow bromegrass appears to be associated with more tillers, rapid remobilization of organic reserves during early regrowth, and allocation of more biomass to leaf tissue than to stems compared to the other two bromegrasses. Variation in regrowth among the species was not associated with expansion of individual leaf area, photosynthetic rates, total organic reserve remobilization, or nitrogen concentration in stem bases. Based on these characteristics, meadow bromegrass is the most suitable species for grazing, and smooth bromegrass the least suitable.

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Dedication

To my mom Deji and father Zhadengbaa for their unconditional love

And

To my wife Numa and daughter Alyssa for their love, support and encouragement

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1.0 Introduction

Canadian cattle numbers reached 15.2 million in 2008, 72% of which were in the provinces of Alberta, Saskatchewan, and Manitoba (CanFax Research Service 2008). This increasing number of cattle increases the demand for, and cost of, feed. As feeding costs are the greatest expenditure in a cattle operation, lowering the feed cost has a major impact on the financial success of operations. Perennial forages are attractive to many producers because perennial forages require fewer inputs, and reduce greenhouse gas (GHG) emissions more than annual cropping systems (Adler et al. 2007). In Saskatchewan, the area seeded to tame hay increased 44% between 2000 and 2008 (Saskatchewan Ministry of Agriculture 2008). Among the many cultivated perennial forage species, bromegrasses (*Bromus*) are valuable and widely cultivated for hay and pasture production. Cultivated bromegrass species can produce high forage yield under the short growing season of western Canada and have excellent nutritive value (Ferdinandez and Coulman 2001). Smooth bromegrass (*Bromus inermis* Leyss.) and meadow bromegrass (*Bromus riparius* Rehm.) are the most commonly cultivated bromegrass species. In recent years, hybrid bromegrass (*B. riparius X B. inermis*) cultivars were developed by hybridizing smooth and meadow bromegrass (Coulman 2004).

Smooth bromegrass is important hay in much of the temperate zone of North America (Vogel et al. 1996), and it is also used for grazing in drier regions of western Canada. Smooth bromegrass was one of the few cool-season grasses to survive the drought of the 1930s, and it has been cultivated widely since then (Casler et al. 2000). Smooth bromegrass also

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tolerates alkaline condition, and extreme temperature variation (Miller 1984); however, it is not tolerant of frequent defoliation (Casler et al. 1998). The value of smooth bromegrass for pasture is also limited by poor seasonal distribution of its yield (Pearen and Baron 1996).

Meadow bromegrass was introduced to Canada in 1980. This grass has become popular in western Canada for pasture and rotational grazing systems in the Dark Brown, Black and Gray Wooded soil zones and in the Brown soil zone under irrigation (Knowles et al. 1993). In comparison to smooth bromegrass, this species offers increased regrowth potential and has more uniform seasonal production, particularly in late summer in western Canada (Knowles et al. 1993). Meadow bromegrass also showed greater potential than smooth bromegrass for early spring grazing in Saskatchewan as determined by an etiolated growth study (Lardner et al. 2003). Its uniform seasonal production and better frost resistance of leaves makes meadow bromegrass suitable for grazing until mid-October (Knowles et al. 1993). In addition, regrowth of meadow brome-alfalfa (*Medicago sativa* L.) mixtures can be used for swath grazing during the winter in western Canada (McCartney 2005). Hybrid bromegrass has shown potential as a dual purpose grass that can be used as hay in spring and as pasture in the summer and fall (Coulman and Knowles 1995).

Regrowth potential differs among the three bromegrass species (Knowles et al. 1993; Coulman 2004), but the morphological and physiological basis for these differences is unclear. Regrowth following defoliation is a complex process and is dependent upon biotic and abiotic factors including response of individual species, phenological stage of growth, the intensity, frequency and duration of defoliation, and environmental condition (Jameson 1963). Knowledge of regrowth traits is critical to sustainable utilization of perennial forage swards. An understanding of regrowth traits can help producers determine the optimum timing of grazing

and can extend the grazing period or increase years of sward use to reduce feed cost. It may also assist forage breeders in selecting superior lines with improved regrowth or persistence under grazing.

The physiology of growth of smooth bromegrass has been extensively studied (Paulsen and Smith 1969; Engel et al. 1987; Ferdinandez and Coulman 2001; Brueland et al. 2003). For meadow and hybrid bromegrasses that have been more recently introduced, studies on growth are limited. A comparative study of these three bromegrass species would provide a better understanding of the morphological and physiological differences that influence growth.

The objectives of this study were to: 1) determine leaf area index, and above- and below-ground dry matter production of three bromegrass species following defoliation at different stages of growth; 2) determine the etiolated growth and stem base nitrogen concentration of three bromegrass species following defoliation at different developmental stages; 3) determine tiller and axillary bud development of three bromegrass species following defoliation at different developmental stages; and 4) compare leaf expansion rate, leaf-to-stem ratio and photosynthetic rate of the three bromegrass species following defoliation.

The hypothesis tested in this study was that meadow bromegrass has superior regrowth ability compared to hybrid and smooth bromegrasses following defoliation because of differences in physiological and morphological characters.

2.0 Literature Review

2.1 Bromegrass species

2.1.1 Origin and characteristics

Meadow bromegrass (*Bromus riparius* Rehm.) a perennial, cool-season grass, is native to southeastern Europe (Tzvelev 1976). It was introduced to western Canada in the early 1980s (Knowles et al. 1993). Meadow bromegrass is decaploid ($2n = 10x = 70$), and its genomic formula is unknown (Armstrong 1990). It has short rhizomes, and its rate of spread is slower than smooth bromegrass (*Bromus inermis* Leyss.). Meadow bromegrass produces many basal leaves that are narrower than those of smooth bromegrass and leaves are pubescent, particularly on the margin of leaves. The inflorescence of meadow bromegrass is an erect, open panicle. The florets of meadow bromegrass are wind-pollinated, and lemmas have awns. Seeds of meadow bromegrass are larger than smooth bromegrass and weigh 5-6g per 1,000 seeds. The plant can grow up to 1.2m at maturity (Knowles et al. 1993).

Smooth bromegrass is also a perennial, cool-season grass, which is native to Europe and northern Asia (Miller 1984). It was introduced to North America in 1884, but was not widely cultivated until the drought of the 1930s. Cultivated smooth bromegrass is an auto-allo-octoploid with $2n = 8x = 56$ and its genomic formula is AAAAB1B1B2B2 (Armstrong 1991). The leaves of smooth bromegrass are broader than meadow bromegrass (Miller 1984). Smooth bromegrass distributes leaves evenly along the stem, and its tillers can grow to more than 1m at maturity.

Smooth bromegrass develops longer rhizomes than meadow bromegrass (Vogel et al. 1996). The inflorescence is an open panicle, which becomes purplish brown at maturity. The lemmas are awnless or have very short awns. Seeds of smooth bromegrass weigh 3-4g per 1,000 seeds (Knowles et al. 1993).

Hybrid populations (B. *riparius* X B. *inermis*) of bromegrass were developed in Canada by crossing meadow bromegrass with smooth bromegrass. An F_1 hybrid ($2n = 9x$) population had 63 chromosomes. The chromosome number varied from $2n = 56\text{-}70$ in the F_2 progeny (Armstrong 1990). Hybrid bromegrass produces both upper and basal leaves, and the leaves have pubescence, which is similar to meadow bromegrass. The leaves of hybrid bromegrass are broader than meadow bromegrass leaves, but narrower than those of smooth bromegrass. Hybrid bromegrass has higher tiller density than smooth bromegrass with a reduced creeping habit (Ferdinandez and Coulman 2000). Knowles was the first hybrid bromegrass cultivar, and released in 2000 (Coulman 2004).

2.1.3 Adaptation

Meadow bromegrass is adapted to cooler and moister areas than smooth bromegrass. These areas include the Black and Gray Wooded soil zones and areas of Dark Brown soil zone, which have relatively higher moisture (Knowles et al. 1993). It is less winter hardy than smooth bromegrass, but leaves of meadow bromegrass have better frost resistance than smooth bromegrass (Limin and Fowler 1987). Meadow bromegrass is also less tolerant to salinity and drought than smooth bromegrass (Knowles et al. 1993). It is resistant to brown-leaf-spot disease, which is caused by *Pyrenophora bromi* (Died.) Drechs. (Berg et al. 1986). This disease causes severe economic losses in smooth bromegrass stands (Knowles et al. 1993); however, meadow bromegrass is susceptible to head smut caused by *Ustilago bullata* Berk. (Gossen and Turnbull

1995). Head smut can reduce seedling establishment and cause losses in seed and forage production.

Smooth bromegrass is adapted to a wide range of soil texture from sandy loam to well-drained silt loam or clay loam (Casler and Carlson 1995). It is seeded widely in Dark Brown and Black soil zones of western Canada (Looman 1983). Smooth bromegrass tolerates alkaline conditions, drought, and extreme temperature variation (Miller 1984). Two major ecotypes, southern and northern, of smooth bromegrass make it adapted to most of the temperate regions of North America. The majority of smooth bromegrass cultivars used in western Canada belong to the northern or intermediate ecotypes (Miller 1984). Smooth bromegrass stands are vulnerable to brown-leaf-spot diseases, but are not infected by head smut.

The hybrid bromegrass, cultivar Knowles, was evaluated over 25 station-years in Canadian provinces and was best adapted to drier areas of the Canadian prairies (Coulman 2004). It is susceptible to brown-leaf-spot disease (Ferdinandez and Coulman 2000).

2.1.4 Major uses

Meadow bromegrass is well adapted to grazing, but it is also sometimes used for hay production in the western Canada (Knowles and Baron 1990; Knowles et al.1993). It regrows faster than smooth bromegrass after defoliation (Knowles et al.1993; Jensen et al. 2001; McCaughey and Simons 1996; Van Esbroeck et al. 1995), particularly under frequent defoliation in the Black or Gray Wooded soils of Canadian prairies (Knowles et al. 1993; McCaughey and Simons 1996). Under irrigation and frequent (six cut) harvesting in northern Utah, meadow bromegrass produced more total dry matter than smooth bromegrass (Jensen et al. 2001). Meadow bromegrass showed greater potential for early spring grazing than smooth bromegrass in Saskatchewan (Lardner et al. 2003). Its uniform seasonal production and leaf frost resistance

makes meadow bromegrass suitable for grazing until mid-October (Knowles et al. 1993). When meadow bromegrass was used in a mixture with alfalfa, the yield distribution was more uniform than smooth brome-alfalfa mixtures, and the yield of meadow brome-alfalfa mixtures equaled or exceeded smooth brome-alfalfa mixtures under frequent harvesting systems (Pearen and Baron 1996). Meadow bromegrass, however, yielded less than smooth bromegrass in a simulated grazing trial under drier conditions in the Dark Brown soil zone (Knowles et al. 1993).

Smooth bromegrass is used primarily for hay. It is most productive under infrequent cutting, relatively high cutting heights, and high N fertilizer (Casler and Carlson 1995). Hay yields of smooth bromegrass are greater than meadow and hybrid bromegrass (Knowles et al. 1993; Ferdinandez and Coulman 2000; Coulman 2004). In addition, the tall and upright tiller growth of smooth bromegrass is suitable for hay harvesting. Smooth bromegrass is also widely used for grazing in drier areas of western Canada, because it can be established easily and has relatively high yield and persistence (McCartney and Bittman 1994; Looman 1983).

Hybrid bromegrass has shown potential as a dual purpose grass that can be used as a hay crop in spring and as a pasture crop in the summer and fall (Coulman and Knowles 1995). Hay yield of hybrid bromegrass is greater than meadow bromegrass, but lower than smooth bromegrass (Coulman 2004). Regrowth of hybrid bromegrass is greater than smooth bromegrass, but slightly less than or similar to meadow bromegrass (Coulman 2004). More animal grazing days were observed on a hybrid bromegrass pasture than meadow and smooth bromegrass pastures in the Black soil zone of Saskatchewan (Thompson et al. 2003).

2.1.5 Forage quality of regrowth

Cultivated bromegrass species have high nutritive value (Carlson and Newell 1985; Knowles et al. 1993). Meadow bromegrass has slightly lower protein and higher fiber

constituents than smooth bromegrass in regowth, but *in vitro* digestibility is similar (Knowles et al. 1993). Initial rejection by cattle sometimes occurs in a meadow bromegrass pasture because of the hairy nature of its leaves (Knowles et al. 1993). Regrowth of meadow bromgrass mainly consists of leaf material, while regrowth of smooth bromegrass and an experimental hybrid bromegrass population has more stem production (Baron et al. 2000). Animal weight gains are similar between smooth and meadow bromegrass during the summer, but they are greater for meadow bromegrass in fall grazing (Knowles et al. 1993). Higher beef production ha^{-1} was reported in a hybrid bromegrass pasture than in smooth and meadow bromegrass pastures (Thompson et al. 2003).

2.2 Effects of defoliation on grasses

2.2.1 Above-ground production

Removal of plant tissues affects a variety of morphological and physiological characteristics in plants. Reduction of above-ground production has been reported in almost all clipping experiments, and the magnitude of reduction was closely associated with frequency, duration, intensity, time of defoliation and response of individual species (Branson 1956; Jameson 1963; Paulsen and Smith 1969; Buwai and Trlica 1977; Mclean and Wikeem 1985; Gold and Caldwell 1989; Turner et al. 2006). Furthermore, responses are likely to change across climates, soil type, soil water content, and soil fertility (Jameson 1963).

Plants can regain their vigor and competitive ability if an adequate amount of time is provided between defoliation events (Buwai and Trlica 1977). In the Aspen-Boreal ecosystem, smooth bromegrass dry matter yield was greater if plants were clipped every four weeks than every two weeks (Donkor et al. 2002). Increasing defoliation frequency reduces herbage yields and dry matter accumulation in many grass species because of depletion of total non-structural

carbon storage or decreased leaf area for photosynthesis (Reed and Dwyer 1971; Buwai and

Trlica 1977; McLean and Wikeem 1985; Turner et al. 2006). The reduction of herbage yield is

more severe when plants are defoliated at a heavy intensity (90% of the current year's growth

removal) than moderate intensity (60% of growth removal) during the same developmental stage

(Buwai and Trlica 1977). Reduced plant injury occurred in rough fescue (*Festuca scabrella* Torr.)

when plants were defoliated to 10 or 15cm stubble height compared to 5cm (McLean and

Wikeem 1985).

Developmental morphology of a perennial grass can be divided into four primary

growth stages: vegetative, stem elongation, reproductive, and seed development and ripening

(Moore and Moser 1995). Differences in availability of meristems for plant growth following

defoliation cause differences in regrowth among growth stages (Briske 1986). Generally, growth

occurs most rapidly from intercalary meristems, followed by newly developed leaf primordia,

and least rapidly from axillary buds (Cook and Stoddart 1953; Hyder 1972; Briske 1986). When

clipped before the end of vegetative growth, regrowth is greater and plant injury is less in many

grasses (Cook 1971; McLean and Wikeem 1985; Brueland et al. 2003; Olson and Richards

1988b); however, plants defoliated after stem elongation or at a more mature developmental

stage exhibit little regrowth (McCarty and Price 1942; Trlica and Cook 1971).

Numerous physiological and morphological differences exist among species in

response to defoliation, and those variations potentially affect regrowth and the period of time

required to resume yield and vigor (Trlica et al. 1977). Buwai and Trlica (1977) stated that

defoliation reduced total non-structural carbon of western wheatgrass (*Agropyron smithii* Rydb.)

while the total non-structural carbon of blue grama (*Bouteloua gracilis* Willd.) was not affected

by defoliation. Caldwell et al. (1981) and Richards and Caldwell (1985) reported that wheatgrass

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(*Agropyron*) species with many similar phenological and physiological traits differed markedly in their ability to produce new tillers following defoliation. Rough fescue and parry oat grass (*Danthonia parryi* Scribn.) produced most forage when cut at a 5cm stubble height with one, two or four cuts in the first year. In the third year, rough fescue produced the greatest yield under a single cut, while parry oat grass produced the most yield in two cuts at heights of 10 and 15cm (Willms 1991).

Though reduction of above-ground production is common in clipping or mowing studies, a number of studies have indicated that above-ground dry matter accumulation may be stimulated by animal grazing (McNaughton 1979; Dyer 1975; Hilbert et al. 1981). They proposed that maximum plant productivity occurs under grazing at an optimal intensity rather than in ungrazed vegetation. The positive effect of grazing on plant productivity has been referred to as herbivore optimization, which is illustrated by the herbivore optimization curve (Hilbert et al. 1981).The grazing animal usually selects particular plant parts rather than harvesting the whole plant as in clipping (McNaughton 1979). A variety of observed mechanisms may account for this increased production by grazing, which include increased photosynthetic rates in remaining leaves (Detling and Painter 1983), greater resource allocation to shoot regrowth (Ryle and Powell 1975), increased tillering from removal of apical dominance (Youngner 1972) or opening of the canopy to allow an increase of light penetration (Laude 1972), conservation of soil moisture by reducing leaf transpiration area (McNaughton 1979), an improved nutrient status in residual tissue, and promotion of growth by hormones in animal saliva (Dyer 1980).

2.2.3 Leaf area development

Rapid production of a new leaves immediately after defoliation is considered a critical trait in response to defoliation, because it allows the plant to assimilate carbohydrates to meet the need of future growth and respiration (Caldwell et al. 1981; McNaughton et al. 1983; Briske 1986). Leaf size, number of leaves per tiller, and tiller density determines the canopy leaf area index (LAI) (Lemaire and Chapman 1996; Lemaire and Agnusdei 2000). Tiller density is considered a key factor for the re-establishment of canopy leaf area following defoliation in many grass species (Caldwell et al. 1981; Van Esbroeck et al. 1995); however, appearance and expansion of leaves following defoliation is also important for leaf area establishment (Davies 1988), and depends on the growth stage and available meristems for regrowth (Briske 1991).

Formation of a leaf primordium begins by rapid cell division in the outermost cell layers of the apical dome, the dermatogen and hypodermis, giving rise to a microscopically visible protuberance (Langer 1972). In vegetative growth, leaf tissues are produced sequentially from leaf primordia, as a chain of phytomers at the level of the individual tiller. Following defoliation at the vegetative stage, rapid leaf development will occur from leaf intercalary meristems, located in narrow zones at the bases of the blade and sheath (Langer 1972), and leaf primordia which remain in the basal position of the tiller (Hyder 1972). After defoliation at the stem elongation stage or after flowering, new tillers from axillary buds become the most important source for canopy leaf area development (Olson and Richards 1988b; Richards et al. 1988). Van Esbroeck et al. (1995) reported that leaf area index (LAI) development of smooth bromegrass is more limited by tiller density than in meadow bromegrass and an experimental hybrid bromegrass population during regrowth in fall.

Leaf appearance and elongation are strongly affected by temperature (Wilhelm and McMaster 1995) and are often expressed in units of thermal time such as growing-degree-days

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(GDD) (Ford 1982). The threshold temperature for leaf appearance of most C_3 species is 3-5 °C (Lemaire and Agnusdei 2000). For a given species, a more or less constant leaf appearance interval can be calculated in terms of GDD (Cruz and Boval 2000). Frank et al. (1985) studied phyllochron (time interval for leaf appearance) of four temperate grass species and found GDD required to achieve a given phyllochron is different among species and among clones within species. Thus, variation of phyllochron among species indicates the different potential for leaf area establishment through individual leaf expansion and appearance. Besides temperature effects, water stress tends to lengthen the leaf appearance in perennial ryegrass (*Lolium perenne* L.) (Volaire et al. 1998), and N and P nutrition increases leaf elongation rate (Gastal et al. 1992).

2.2.4 Net photosynthesis

Photosynthesis is the process of converting light energy to chemical energy and storing it in the bonds of sugar. In a C_3 species, the process of photosynthesis primarily takes place in leaves, specifically in chloroplasts of mesophyll cells. Photosynthetic rates of leaves is related to chlorophyll content, stomatal conductivity, and leaf photosynthetic N-use-efficiency (the rate of photosynthesis per unit of leaf N); therefore, photosynthetic rates of leaves vary among species (Pons and Westbeek 2004).

For a given species, defoliation alters the age structure of leaves within the plant canopy and modifies plant photosynthetic capacity (Briske 1991). Leaves of defoliated plants may have higher photosynthetic rates than relatively older foliage on undefoliated plants (Caldwell et al.1981; Wallace et al. 1984), because plant leaves generally exhibit maximum photosynthetic rates at the time of full expansion, and decline thereafter (Caldwell 1984).

An increase in photosynthetic rates of foliage after partial defoliation has been reported as a mechanism to compensate for defoliation (McNaughton 1979). Following partial

defoliation, the rate of photosynthesis declined immediately in the remaining undamaged leaves followed by an increase, or less rapid decline with age than the photosynthetic rate in similar leaves from undefoliated plants (Gifford and Marshall 1973; Detling and Painter 1983; Nowak and Caldwell 1984). This photosynthetic rate enhancement has been attributed to increased leaf stomatal conductance in the remaining leaves of the defoliated plant (Gifford and Marshall 1973) or increased N-use-efficiency in leaves (Caldwell et al. 1981; Wallace et al. 1984). In addition, increasing light penetration to the shaded leaves following partial defoliation also plays a role in increase of photosynthesis (McNaughton et al. 1983).

Enhanced photosynthetic rates have been observed in numerous studies (Gifford and Marshall 1973; Detling and Painter 1983; Nowak and Caldwell 1984), but gross photosynthesis of the sward is a function of total leaf area and photosynthetic rates of individual leaves (Briske 1991). Brougham (1958) suggested that each species has a critical LAI, and gross photosynthesis of a grass sward may be more closely related to LAI rather than photosynthetic rates of individual leaves. Parsons et al. (1983) reported that gross photosynthesis of the sward was greater in a sward of perennial ryegrass with high LAI than a sward with lower LAI.

2.2.4 Tiller development

Tillers arise from axillary buds, rudimentary apical meristems that form in the deeper (sub-hypodermal) layers of the apex during the early development of leaf primordia (Langer 1972; Jewiss 1972). Following a juvenile period of development, primary tillers are potentially capable of initiating secondary tillers from their own axillary buds at the stem bases (Briske 1991). Further development of these buds can produce a hierarchy of tillers that are connected by a complex system of vascular tissue (Langer 1972). Tiller recruitment in temperate, perennial grasses is most prevalent in the spring and fall, yielding two tiller generations annually (Butler

and Briske 1988). Tiller development following defoliation, however, is unique for each grass species. Caldwell et al. (1981) and Richards and Caldwell (1985) reported that wheatgrasses (*Agropyron*) with similar phenology and physiological traits differed markedly in their ability to produce new tillers following defoliation. In smooth bromegrass, tillering ceased at the stem elongation stage, and did not resume again until anthesis stage unless apical dominance was removed by defoliation (Eastin et al. 1964).

Tiller initiation from the axillary bud is a complex biochemical process under control of hormonal, environmental and nutritional factors (Langer 1972). Activation of axillary buds usually occurs after tiller flowering or senescence, or in response to defoliation during internode elongation in many grasses (Branson 1953; Paulsen and Smith 1969; Jewiss 1972; Olson and Richards 1988a). Traditionally, the concept of apical dominance was used to explain tiller initiation in perennial grasses (Murphy and Briske 1992). Apical dominance refers to the control exerted by the apical portion of the shoot, which includes the apical meristem and young leaves, on axillary bud growth following bud formation (Cline 1991). Five major hypotheses of apical dominance had been advanced for dicots; however, a hormonal mechanism was more widely accepted in grasses (Murphy and Briske 1992). The hormonal hypothesis indicated that auxin transported down the stem from the apical meristem blocks cytokinin synthesis or utilization in axillary buds, thereby inhibiting growth (Phillips 1975). Murphy and Briske (1992) in their review indicated that hormones other than auxin and cytokinin such as abscisic acid (ABA), gibberellins (GA) and ethylene may be involved in apical dominance.

The nutrition hypothesis is based on the concept of resource-sink relationship in which apical dominance is maintained by the internal competition among buds for nutrients (Gregory and Veale 1957). While the nutrient requirements of the existing shoot meristems

exceed the supply rate, lateral bud inhibition is maintained by nutrient deprivation. Once the nutrient supply rate exceeds the demand rate of existing meristems, the increased nutrient concentration in the shoot stimulates lateral bud outgrowth (Gregory and Veale 1957). Apical decapitation removes the metabolic sink, thereby allowing resources to be redirected to lateral bud outgrowth, an alternative interpretation to apical dominance (Gregory and Veale 1957). Briske and Derner (1998) found inconsistencies with each mechanism and concluded that searching for a sole regulatory mechanism for buds regulation may have limited success. Tomlinson and O'Connor (2004) developed an integrated model for bud release considering hormonal, nutritional control and grass photosensitivity to red-far-red light ratio. They concluded that hormonal control (auxin: cytokinin ratio) is determined by red-far-red light ratio (auxin production and export from shoot) and soil N (cytokinin production).

Though it was generally believed that tillers were initiated in response to removal of apical meristems after internode elongation in many grasses, tillering can occur in response to defoliation even if apical meristems have not been removed (Butler and Briske 1988). Selective removal of apical meristems, while leaves remain intact, does not stimulate tiller initiation in temperate grasses (Richards et al. 1988). Other studies have indicated that tiller initiation was suppressed by defoliation due to the senescence of tillers after apical removal and a lower amount of energy for bud growth (Branson 1956; Ellison 1960; Jameson 1963). Jameson (1963) suggested that differences in plant response to defoliation are perhaps not due to removal of the apex, but instead are related to plant vigor or number of vegetative stems. Murphy and Briske (1992) suggested that defoliation may simply alter the timing of tiller initiation rather than increase the total number of tillers during the long-term.

In general, following defoliation in early spring before internode elongation, existing tillers continue to elongate to ensure tiller density (Jewiss 1972), and new tillers can also arise from axillary buds (Paulsen and Smith 1969). When defoliation occurs during or immediately after internode elongation, apical meristems are often removed (Hyder 1972; Jewiss 1972), and tiller development comes from axillary buds (Carlson and Newell 1985). Slow activity of axillary buds at the stage of internode elongation usually results in slow tiller development in many grasses (Paulsen and Smith 1968; Langer et al. 1964; Olson and Richards 1988a).

Several environmental factors influence tiller initiation. In cool-season grasses, the optimum temperature for tillering is relatively low and varies among species (Langer 1972). High temperatures inhibit tillering because of high respiration rates and lower soluble carbohydrate concentrations in the plant (Langer 1972). The ability of grasses to produce tillers is also sensitive to changes in light intensity. Tiller density increases with increasing light intensity (Langer 1972; Ashmun and Pitelka 1984). Greater soil moisture availability increases tillering in late spring and summer (Cook et al.1958). Tiller production is generally enhanced by increasing the supply of N, P, and K, with N generally considered to be the most important nutrient (Langer et al.1964; Shaver et al. 1986).

2.2.5 Organic reserves and regrowth

Vast quantities of carbon (C) and nitrogen (N) reserves accumulate in specialized storage organs of plants (Volenec 2007). Carbohydrate reserves are important for winter survival, early spring growth initiation and regrowth after defoliation when photosynthetic production is inadequate (Brown and Blaser 1965; White 1973).

The terms "total available carbohydrate", "total non-structural carbohydrates" and "water soluble carbohydrate" have been used to describe the carbohydrate reserves in many

studies (Weinmann 1948; Smith 1969; Turner et al. 2006).   Non-structural carbohydrates include reducing sugars (glucose and fructose), non-reducing sugars (sucrose and fructosans), and starches (White 1973). Predominant carbohydrate reserves stored by temperate grasses are fructosans and sucrose (Weinmann 1948; Okajima and Smith 1964), which are stored in stem bases, stolons, rhizomes and corms (Baker and Garwood 1961; Reynolds and Smith 1962). Non-structural carbohydrates in the roots of grasses are likely not used directly in regrowth following defoliation (Marshall and Sagar 1965); however, a more recent study of prairie grass (*Bromus willdenowii* Kunth.) suggests that this species is reliant on root carbon reserves in addition to stubble reserves to meet energy needs before leaf area establishment (Turner et al. 2007).

Seasonal carbohydrate cycles are related to developmental stages of growth. In many temperate grasses, the lowest amount of carbohydrates occurs after early spring growth initiation, and the maximum level is reached at anthesis or seed-shattering (Reynolds and Smith 1962; Eastin et al. 1964; Trlica and Cook 1972; Menke and Trlica 1981). The magnitude of seasonal fluctuation of carbonhydrate reserves was related to variation of temperature, soil moisture and soil nutrients (White 1973).

Reduced plant carbohydrate reserves following defoliation have been associated with carbon translocation to regrowing tissues and respiration (Reynold and Smith 1962; Davidson and Milthorpe 1966b; White 1973; Trlica and Cook 1971, 1972; Buwai and Trlica 1977; Gonzalez et al. 1989). Steady-state ^{13}C labeling was used to investigate the use of remobilized and currently assimilated C into the leaf and root growth zones of perennial ryegrass after a single defoliation. It was estimated that 50% of the carbon was derived from remobilization during the first three days of regrowth, falling to 10% after five days (De Visser et al. 1997). In another study on perennial ryegrass, Donaghy and Fulkerson (1997) estimated that

remobilization of stored carbohydrate contributed 33% and current assimilation 66% to regrowth after defoliation. Several other studies have examined the contribution of carbohydrate reserves to regrowth (May 1960; Caldwell et al. 1981; Davidson and Milthorpe 1966; Richards and Caldwell 1985), and concluded that carbohydrate reserves were important for only a few days of regrowth immediately after a severe defoliation.

N reserves are also an important fraction of plant organic reserves and are essential to regrowth (Ourry et al. 1994; Dilz 1966). A ^{15}N study showed that a significant amount of N was remobilized during regrowth in annual soft chess grass (*Bromus mollis* L.) and perennial ryegrass (Phillips et al. 1983; Ourry et al. 1988, 1990). N was remobilized from roots and stubbles to growing leaves following a single defoliation with the majority of N coming from stubble (Ourry et al. 1988). After two weeks of regrowth, 40-60% of N in regrowing leaves of perennial ryegrass came from remobilization (Ourry et al. 1990). Amino N seems to be the most readily available form of N (Ourry et al. 1988), and protein N is the largest storage form of N (Ourry et al. 1988); however, other studies questioned the significance of N remobilization and suggested that N uptake by roots was more important than remobilization from roots in supplying N to shoot regrowth of some grasses (Thornton and Millard 1996). The degree to which N remobilization contributes to regrowth can depend on internal concentration and external supply (Skinner et al. 1999).

2.2.6 Root growth and biomass

Below-ground development of grasses is often suppressed by shoot defoliation (Weaver and Zink 1946; Jameson 1963). The magnitude of root biomass reduction was much greater than reduction in herbage yield in rough fescue after defoliation of only 20 % (12.5cm stubble height) of above-ground growth in greenhouse conditions (Johnston 1961). Deterioration

of roots following clipping was most rapid near the root tips where root apical meristems and elongation zones are located (Jameson 1963). Root elongation may cease within 24 hours following a severe defoliation (Crider 1955; Ryle and Powell 1975). Reduction in the number of roots initiated and elongated became progressively more severe with increasing frequency and intensity of defoliation (Crider 1955; Evans 1973).

2.2.7 Resource allocation

In grasses, resource allocation is changed by defoliation. For example, in barley (*Hordeum vulgare* L.), the relative proportion of photosynthetic carbon allocated to roots decreased following defoliation, and the proportion of carbohydrate allocation to regrowing shoots increased (Ryle and Powell 1975). The sequence of priority for allocation of water soluble carbon following defoliation was in order of leaf growth, roots and tiller formation and development in prairie grass (Turner et al. 2007). In perennial ryegrass, the first priority following defoliation was to grow new leaf material to restore photosynthetic capacity, while the second and third priorities were to replenish water soluble carbon reserves of stem bases and to initiate new tillers, respectively (Donaghy and Fulkerson 1998).

In addition, greater resource allocation to shoot development following defoliation was reported for more rapidly regrowing species compared to slow-growing species (Caldwell et al. 1981; Richards 1984). For example, defoliation-tolerant crested wheatgrass (*Agropyron desertorum* Schult.) allocated more C to shoot than root systems when defoliated, whereas defoliation sensitive bluebunch wheatgrass (*Agropyron spicatum* Scribn.) continued to allocate more C to the root system. Guitian and Bardgett (2000) found similar responses for red fescue (*Festuca rubra* L.) and crested dog's tail grass (*Cynosurus cristatus* L.) and defoliation-sensitive sweet vernalgrass (*Anthoxanthum odoratum* L.).

3.0 Common Methodology and Meteorological Data

3.1 Plant materials

Three bromegrass species were used in this study including meadow bromegrass cv. Fleet, smooth bromegrass cv. Carlton, and hybrid bromegrass cv. Knowles. All three cultivars were developed at the Saskatoon Research Centre of Agriculture and Agri-Food Canada (AAFC). Fleet, released in 1987, was selected for seed production, reduced awn development, and reduced seed shattering, and further selections were carried out based on ploidy, growth habit, and floret fertility (Alderson and Sharp 1995). Carlton, a northern ecotype, was released in 1961, and was selected for high forage and seed yields, and resistance to brown-leaf-spot disease (Alderson and Sharp 1995). Hybrid bromegrass, cv. Knowles, was released in 2000. It was selected from hybrid populations generated by crossing meadow bromegrass (cv. Fleet and cv. Paddock) and smooth bromegrass (cv. Signal). Several generations of recurrent selection were conducted for increased vigor, improved floret fertility, good regrowth, and reduced creep (Coulman 2004).

3.2 Field plot location and experimental design

Field plots were located at the AAFC Research Farm ($52^007'$ N, $106^038'$ W) in Saskatoon, Saskatchewan. Field studies were conducted during the summers of 2006 and 2007 in two trials consisting of meadow, smooth, and hybrid bromegrasses, which were established in 2004 and 2006, respectively. The soil was a Dark Brown Chernozem (Head 1979). Each trial

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was established in a four replicate, randomized-complete-block-design with each plot consisting of four, 6m long rows, spaced 30cm apart and seeded at a rate of 100 seeds m^{-1}.

3.3 Meteorological data and growing-degree-day determinations

Rainfall was recorded for the months of April, May, June and July in both years (Fig 3.1). Rainfall during the experiments was 225 mm in 2006 and 147.5 mm in 2007 (Fig 3.1). Total cumulative growing-degree-days (GDD) were 1,786 in 2006 and 1,704 in 2007 (Fig 3.2). Mean daily temperature exceeded the base temperature (0°C) for five consecutive days on 4 April 2006 and 12 April 2007. Field studies were completed 2 August 2006 and 27 July 2007. Thus, these were the starting and ending dates for measurement of GDD, which were calculated according to equation 3.1 (Frank and Hofmann 1989):

GDD = $\sum$ (daily maximum temperature + daily minimum temperature) / 2   (3.1)
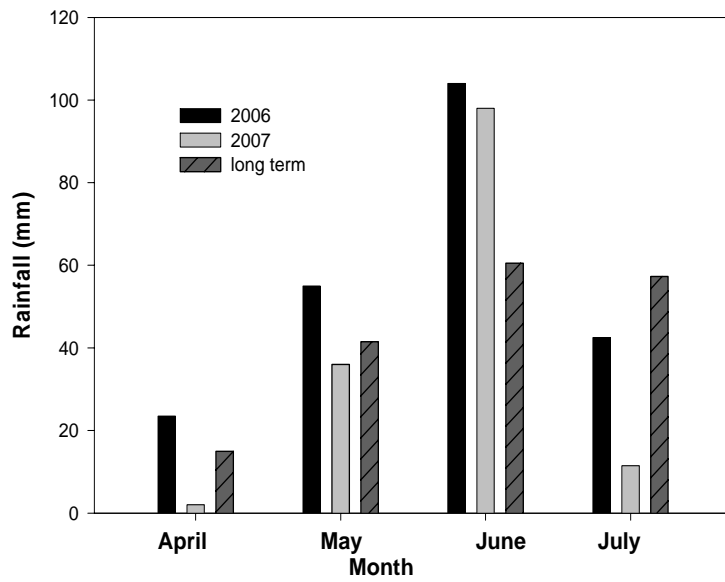
Figure 3.1 Monthly rainfall received during the studies in 2006 and 2007 and the long-term average (1970-2000) at Saskatoon, Saskatchewan.
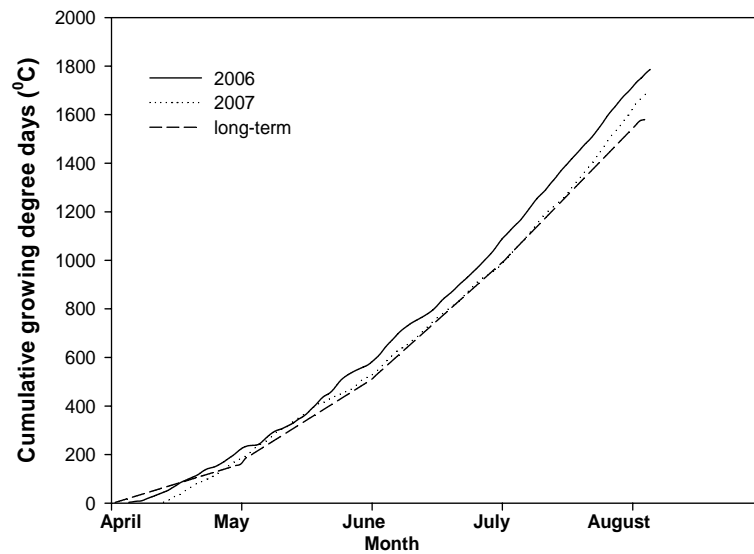
Figure 3.2 Cumulative growing-degree-days (GDD) during the field studies in 2006 and 2007 and the long-term average (1970-2000) at Saskatoon, Saskatchewan.

4.0 Above-and Below-Ground Biomass Production and Leaf Area Index of Three Bromegrass (*Bromus*) Species in Response to Defoliation at Different Developmental Stages

Abstract   Bromegrasses (*Bromus*) are cultivated for pasture and hay in western Canada. The objective of this study was to determine above-and below-ground biomass and leaf area index (LAI) of meadow bromegrass (*Bromus riparius* Rehm.), smooth bromegrass (*Bromus inermis* Leyss.) and hybrid bromegrass (*B. riparius X B. inermis*) after defoliation. The study was conducted in 2006 and 2007 in Saskatoon ($52^0$07' N, $106^0$38' W), Saskatchewan on a Dark Brown Chernozem soil. Plants were clipped to a 5cm height at the vegetative and stem elongation stages of growth, and an undefoliated control was included. Regrowth was similar (521 g m^{-2}) among the three species when defoliated at the vegetative stage, but meadow and hybrid bromegrass produced 42% greater regrowth than smooth bromegrass following defoliation at the stem elongation stage. Compared to undefoliated plants, below-ground biomass was reduced 38% following defoliation. Meadow and hybrid bromegrass produced similar (4863 g m^{-3}) below-ground biomass, which was 40% greater than smooth bromegrass (2923 g m^{-3}). LAI of all three bromegrasses increased linearly with days of regrowth ($r^2 \geq 0.88$), and LAI was greatest in meadow bromegrass (4.0, 3.3), intermediate in hybrid bromegrass (3.6, 2.7), and least in smooth bromegrass (3.1, 2.2) following defoliation at the vegetative and stem elongation stages, respectively. Smooth bromegrass is sensitive to defoliation at the stem elongation stage, thus, higher defoliation height is necessary at this growth stage.

4.1 Introduction

Meadow bromegrass (*Bromus riparius* Rehm.) is recognized for its rapid regrowth after defoliation and is mainly used for pasture (Knowles et al. 1993). Smooth bromegrass (*Bromus inermis* Leyss.) is generally considered to be best of the bromegrasses for hay production (Casler and Carlson 1995) because of its uniform leaf arrangement, upright tillering and greater dry matter yield. The hybrid bromegrass (*B. riparius X B. inermis*) cultivar Knowles was recently developed by hybridizing of smooth and meadow bromegrass (Coulman 2004), and has potential for both hay or pasture use. Variation in regrowth of these three species in the fall or after frequent defoliation has been reported (Knowles et al. 1993; Van Esbroeck et al. 1995; Baron et al. 2000); however, little information is available concerning regrowth and canopy LAI development following defoliation at different stages of growth in these bromegrass species.

The developmental stage of growth at defoliation influences the regrowth dry matter accumulation in cool-season grasses because of differences in available meristems (Cook 1971; Olson and Richards 1988b; Brueland et al. 2003). Generally, regrowth occurs most rapidly from intercalary meristems, followed by newly developed leaf primordia, and least rapidly from axillary buds (Cook and Stoddart 1953; Hyder 1972; Briske 1986); however, available meristems after defoliation may vary among species because of species differences in the number of non-elongated tillers and axillary buds.

Defoliation usually causes an immediate reduction of root growth (Crider 1955; Davidson and Milthorpe 1966), which in turn restricts water and nutrient uptake from the soil (Clement et al. 1978). Depression of root production by clipping is common (Weaver and Zink 1946; Ellison 1960) with the magnitude of reduction varying among species (Weaver and Zink 1946). Grasses with rapid shoot regrowth appear to reduce root growth following defoliation

more than species with slow regrowth because relatively more resources are allocated to shoot regrowth (Richards 1984).

Rapid replacement of leaf area following defoliation is critical to achieve a positive carbon balance to support further plant development (Davies 1988). Leaf area is determined by leaf size, leaves tiller^{-1}, and tiller density in grasses (Lemaire and Chapman 1996). In fall regrowth, leaves tiller^{-1} and leaf area tiller^{-1} were greater in smooth bromegrass than meadow bromegrass, while an experimental hybrid bromegrass population was similar to smooth bromegrass (Van Esbroeck et al.1995). Leaf appearance rate tiller^{-1} following grazing was also higher in smooth bromegrass than meadow bromegrass (Lardner et al. 2003). Smooth bromegrass has greater LAI than meadow and hybrid bromegrass in undefoliated swards (Ferdinandez and Coulman 2000); however, smooth bromegrass LAI development in the fall was slower than the other two bromegrasses because of fewer tillers (Van Esbroeck et al. 1995).

This study was designed to test the hypothesis that meadow bromegrass has greater above-and below-ground biomass than smooth bromegrass and hybrid bromegrass following defoliation at various developmental stages, and it also increases leaf area index (LAI) more rapidly after defoliation. The objective of this study was to determine above-and below-ground biomass and LAI following defoliation at the vegetative and stem elongation stages of three bromegrass species.

4.2 Materials and methods

4.2.1 Data collection

4.2.1.1 Above-ground biomass

Above-ground biomass was determined in two 15 x 20cm quadrats randomly placed over rows in each plot after defoliation to 5cm at the vegetative (first leaf collared) and stem elongation (second node visible) stages (Moore and Moser 1995). Undefoliated controls were included in each plot. In each year, regrowth was harvested after a similar number of GDD had accumulated following each developmental stage of defoliation (Table 4.1). Above-ground biomass was also harvested in quadrats in the undefoliated controls. Harvested biomass was dried at 60°C for 48h and weighed.

Table 4.1. Date of defoliation for above-ground biomass and the corresponding cumulative growing-degree-days (GDD) between defoliation and measurement for three bromegrasses during the summers of 2006 and 2007 at Saskatoon, Saskatchewan.

	Date of defoliation		Date of measurement		GDD (°C)	
	2006	2007	2006	2007	2006	2007
Vegetative stage						
all three bromegrasses	10 May	9 May	1 Aug	13 Jul	1465	964
Stem elongation stage						
meadow bromegrass	19 May	25 May	28 Jul	24 Jul	1271	1049
smooth bromegrass	22 May	31 May	1 Aug	27 Jul	1304	1045
hybrid bromegrass	22 May	26 May	1 Aug	24 Jul	1304	1041

4.2.1.2 Below-ground biomass

The treatments for below-ground biomass determinations were the same as for above-ground biomass, except that an additional defoliation at a reproductive stage (inflorescence emerged) was included. Soil cores were collected with an 8cm diameter soil corer to a depth of 20cm after 46d of regrowth (30d of regrowth for the reproductive stage defoliation) from the defoliated plots and undefoliated control. Soil cores were soaked in warm water for 2-3d and then placed on three layers of mesh screens of 5, 1, 0.5mm diameter, which were placed over a pail, and washed

with water under high pressure. Roots that remained on the three screens were collected and combined. Washed samples were dried in the oven at 80°C for 48h and weighed.

4.2.1.3 Leaf area index

Two 1m long parallel rows from each plot were defoliated to a 5cm stubble height at the vegetative or stem elongation stage, and then allowed to regrow for 43-46d depending on the number of GDD that had accumulated following defoliation (Table 4.2). Two undefoliated controls in each plot were also selected for the leaf area index (LAI) measurement. LAI was indirectly determined using the LAI-2000 plant canopy analyzer (Li-Cor, Inc. Nebraska, USA) at 15, 28 and 46d (43d for smooth bromegrass and 45d for hybrid bromegrass in 2007) after defoliation. An opaque mask was used to reduce the field of view to 45° to reduce measurement error. All LAI measurements were obtained between 1500h-1800h to avoid direct sun. One above-canopy reading (A) and four below-canopy readings (B) along diagonal transects between the rows were made and repeated for one complete LAI measurement. Two values were recorded for each treatment plot. A direct estimate of LAI was also made in the undefoliated swards of the three bromegrass species to estimate actual leaf area (see Appendix B). The actual LAI and LAI-2000 estimations showed a similar ranking for the three species.

Table 4.2. Date of defoliation of three bromegrass species at the vegetative and stem elongation stages and corresponding cumulative growing-degree-days (GDD) at the final of three leaf area index (LAI) measurements.

	Date of defoliation		Total GDD (46d[†])		
	2006	2007	2006	2007	
Vegetative stage					
all three bromegrasses	11 May	15 May	708	684	
Stem elongation stage					
meadow bromegrass	19 May	24 May	761	758	
smooth bromegrass	23 May	31 May	784	770[1]	
hybrid bromegrass	23 May	26 May	784	771[2]	

†Number of growth days following defoliation.
[1]Final LAI was determined at 43 days.
[2]Final LAI was determined at 45 days.

4.2.2 Statistical analysis

Data were analyzed as a split plot in a randomized-complete-block-design (RCBD) with 4 replications using SAS.9.1.3 Proc Mixed model (SAS Institute Inc. 2003) to determine the effects of species, defoliation, and their interaction on above-and below-ground biomass. Species, defoliation, and their interaction were considered fixed effects, and year, block and block x species were considered as random effects in the model. The two sub-samples of above-ground biomass and LAI from each plot were averaged, and means were used in the analysis. Values of above-and below-ground biomass were further compared among species in each defoliation treatment. Below-ground biomass was also compared for different defoliation treatments within the same species. When analysis of variance (ANOVA) indicated significant differences (P≤0.05), means were separated using the least square means comparison. Regression analysis for LAI on days after defoliation was conducted in each species for the different defoliation treatments.

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4.3 Results

4.3.1 Above-ground biomass

The species (P=0.21) main effect and the species x developmental stage of defoliation (P=0.27) interaction effect on above-ground biomass were not significant, but developmental stage of defoliation had a significant effect (P<0.01). Overall, regrowth after defoliation was 64% less than that of the undefoliated control, and regrowth was 46% greater when defoliated at the vegetative stage than at the stem elongation stage.

Above-ground biomass was further compared among species at each defoliation stage (Table 4.3). With no defoliation, hybrid bromegrass produced 14% greater biomass than meadow and smooth bromegrass, but meadow bromegrass and smooth bromegrass did not differ. Regrowth was not different among the three species when defoliated at the vegetative stage, averaging 521 g m^{-2}. Following defoliation at the stem elongation stage, meadow and hybrid bromegrass produced 42% greater regrowth than smooth bromegrass, but no difference was found between meadow bromegrass and hybrid bromegrass.

Table 4.3. Above-ground biomass (g m^{-2}) of three bromegrass species after 60 days of growth in undefoliated control or following defoliation to 5cm in a field study conducted in 2006 and 2007 at Saskatoon, Saskatchewan.

Species	Defoliation treatment		
	Undefoliated control	Defoliated at vegetative stage	Defoliated at stem elongation
	---------------------------------- g m^{-2} ----------------------------------		
meadow bromegrass	1039 b[z]	557 a	324 a
smooth bromegrass	1072 b	493 a	217 b
hybrid bromegrass	1224 a	512 a	295 a
P	0.03	0.48	0.01
SEM[y]	203	85	74

[z] Means within a column with the same letter (*a-b*) are not significantly different (P≤0.05).
[y] Standard error of the mean.

4.3.2 Below-ground biomass

Species (P<0.01) and developmental stage of defoliation (P<0.01) affected below-ground biomass, but the interaction between these two factors (P=0.90) was not significant. Overall, meadow bromegrass had the greatest below-ground biomass, whereas smooth bromegrass had the least. Undefoliated plants produced 38% more below-ground biomass than defoliated plants, but below-ground biomass did not differ among the defoliation treatments.

Data were further analyzed for each defoliation treatment to compare the below-ground biomass among species (Table 4.4). Meadow bromegrass produced 30% more below-ground biomass than smooth bromegrass in the undefoliated control and was 40-51% greater after defoliation. Below-ground biomass of meadow bromegrass was similar to hybrid bromegrass except meadow bromegrass produced 28% more below-ground biomass when defoliated at the stem elongation stage. Below-ground biomass of hybrid bromegrass was 29 and 36% greater than smooth bromegrass following defoliation at the stem elongation and reproductive stages, respectively. Compared to undefoliated plants, the percent reduction of below-ground biomass was greater in smooth bromegrass after defoliation than meadow and hybrid bromegrasses, ranging from 44-51% (Table 4.4). The reduction of below-ground biomass compared to undefoliated plants was similar between meadow and hybrid bromegrass except the reduction was less in meadow bromegrass when defoliated at the stem elongation stage.

Table 4.4. Below-ground biomass (g m^{-3}) of three bromegrass species after 60 days growth in an undefoliated control or following defoliation to 5cm in a field study conducted in 2006 and 2007 at Saskatoon, Saskatchewan.

Species	Undefoliated control	Defoliated at vegetative stage	Defoliated at stem elongation stage	Defoliated at reproductive stage	P	SEM
			--- g m^{-3} ---			
meadow bromegrass	7980 a[Z] E[X]	4560 a F (-43)[‡]	5650 a F (-29)	6300 a EF (-21)	0.006	660
smooth bromegrass	5610 b E	2740 b F (-51)	2870 c F (-49)	3160 b F (-44)	0.002	450
hybrid bromegrass	6430 ab E	3660 ab F (-43)	4050 b F (-37)	4960 a F (-23)	0.001	950
P	0.03	0.01	<0.01	<0.01		
SEM[y]	600	720	510	700		

[Z] Means within a column with the same lower case letter (*a-c*) are not significantly different (P≤0.05).

[X] Means within a row with the same upper case letter (*E-F*) are not significantly different (P≤0.05).

[y] SEM= standard error of the mean.

[‡] Percentage of reduction in below-ground biomass compared to undefoliated control.

4.3.3 Leaf area index (LAI)

The LAI of meadow bromegrass increased linearly with time in the undefoliated treatment; however, the linear models to predict LAI from days of growth for smooth bromegrass and hybrid bromegrass were not significant (P≥0.05), because less rapid development of LAI occurred between Days 28 and 46 than between Days 15 and 28 for both species. The LAI reached 4.2, 4.3 and 4.1 in meadow, smooth and hybrid bromegrasses, respectively, after 46 days growth of the undefoliated treatment. Following defoliation at the vegetative or stem elongation stages, all three bromegrasses increased LAI in a linear fashion (Fig. 4.3b-c). After 46 days of regrowth, maximum LAI of 4.0, 3.1 and 3.6 were reached in meadow, smooth, and hybrid bromegrasses, respectively, after defoliation at the vegetative stage, and 3.3, 2.2 and 2.7, respectively, after defoliation at the stem elongation stage.
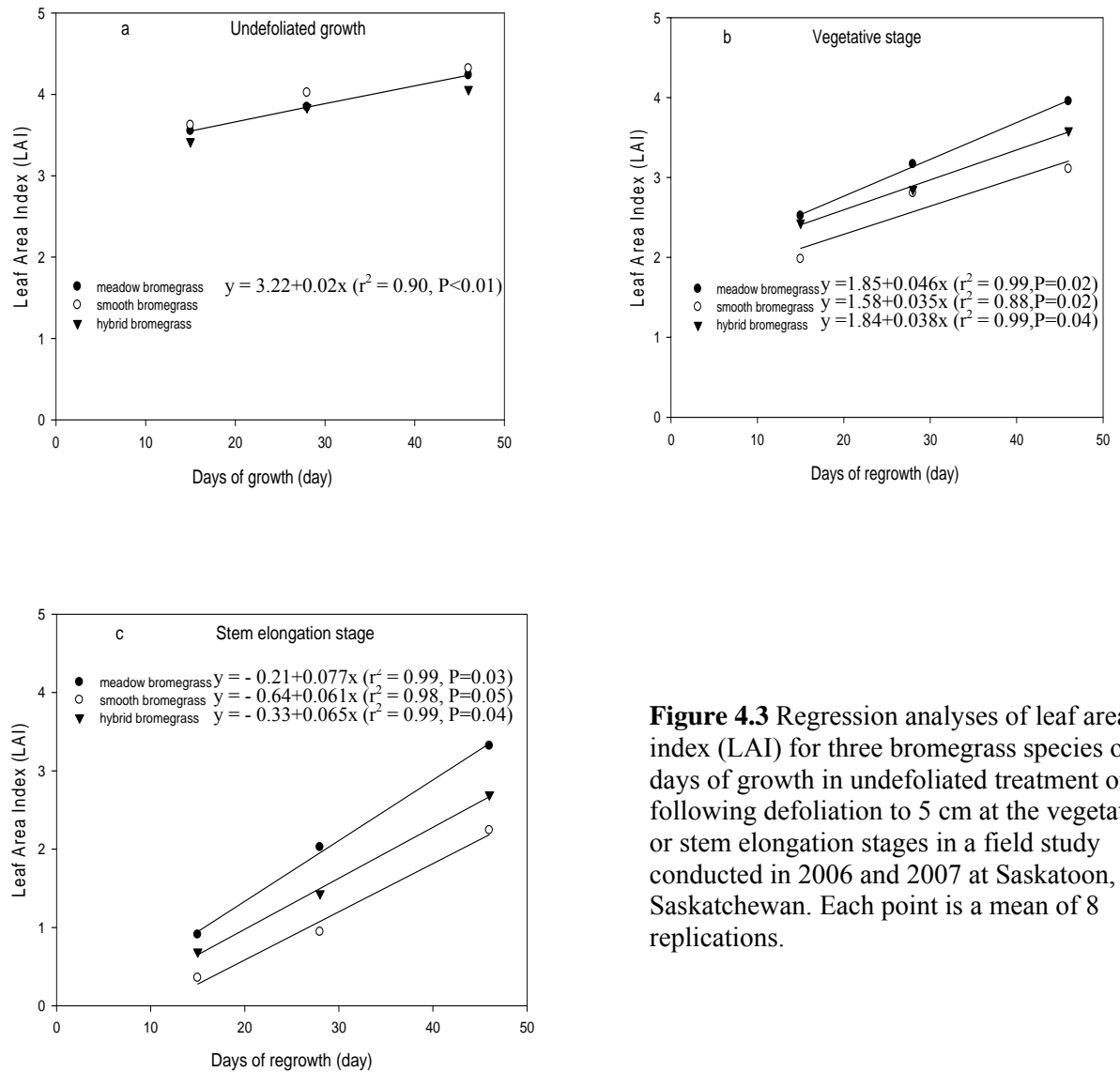
Figure 4.3 Regression analyses of leaf area index (LAI) for three bromegrass species on days of growth in undefoliated treatment or following defoliation to 5 cm at the vegetative or stem elongation stages in a field study conducted in 2006 and 2007 at Saskatoon, Saskatchewan. Each point is a mean of 8 replications.

4.4 Discussion

In the present study, regrowth after defoliation at the vegetative stage was similar among the three bromegrasses. Smooth bromegrass, however, produced significantly less regrowth than meadow and hybrid bromegrass following defoliation at the stem elongation stage.

Van Esbroeck et al. (1995) and Baron et al. (2000) also reported less regrowth of smooth bromegrass than meadow bromegrass and an experimental hybrid bromegrass population following defoliation at the reproductive stage. Thus, difference in regrowth among the three species may only occur when defoliated after stem elongation. Differences in the number of unelongated tillers after the stem elongation stage may provide an explanation for the variation in regrowth among these species. Meadow bromegrass has a greater number of unelongated tillers than smooth bromegrass when defoliated after stem elongation (Knowles et al. 1993). Hybrid bromegrass was intermediate for this trait (see Chapter 5). Elongated tillers die after a severe defoliation (Davies 1976), and regrowth following defoliation is from existing vegetative tillers or basal axillary buds. Regrowth of existing vegetative tillers is a relatively faster process compared to growth of axillary buds (Cook and Stoddart 1953; Hyder 1972; Briske 1986). Bonesmo (2000) also reported that the percentage of non-elongated tillers is positively related to daily maximum regrowth rate in meadow fescue (*Festuca pratensis* Huds.) and timothy (*Phleum pratense* L.) following defoliation at different developmental stages.

Meadow bromegrass produced more below-ground biomass than smooth bromegrass after defoliation, and hybrid bromegrass was intermediate for this trait. Thus, meadow bromegrass maintains relatively more roots, while producing large amounts of above-ground regrowth. Mapfumo et al. (2002) stated that meadow bromegrass produced greater root dry matter than smooth bromegrass under low intensity grazing, but root dry matter was similar under medium or high intensity grazing. Plants with a large root system or high root proliferation rates can occupy greater soil volumes and gather a greater share of soil resources (Caldwell et al. 1987). The higher below-ground biomass in meadow bromegrass suggests that this species can access greater amounts of soil nutrients than smooth bromerass after defoliation. Richards (1984)

reported that *Agropyron* species with rapid regrowth appeared to reduce root growth following

defoliation more than species with slow regrowth because they allocated relatively more

resources to shoot regrowth. Defoliation reduced below-ground biomass of the three

bromegrasses compared to undefoliated plants, but the magnitude of the reduction relative to

undefoliated plants was greatest in smooth bromegrass. Reduction in root growth and

productivity are considered to be detrimental to the survival and competitive ability of defoliated

plants (Crider 1955; Jameson 1963). This greater reduction of below-ground biomass in smooth

bromegrass likely reduced its recovery potential after defoliation.

Rapid production of a new canopy after defoliation is considered a critical trait in

recovery from defoliation because plants rapidly re-establish a positive carbon balance from

photosynthesis (Caldwell et al. 1981; McNaughton et al. 1983; Briske 1986). In the present study,

meadow bromegrass had the greatest LAI, and smooth bromegrass had the lowest LAI following

defoliation. Van Esbroeck et al. (1995) found that LAI was greater for meadow bromegrass and

an experimental hybrid bromegrass population than for smooth bromegrass in fall regrowth.

They suggested that low tiller density was the key factor limiting the LAI development of

smooth bromegrass at this stage. In the present study, tiller density was also lower in smooth

bromegrass (see Chapter 5).

The linear model did not fit to predict LAI development of smooth bromegrass and

hybrid bromegrass in the undefoliated control. Senescence of lower leaves noticed in

undefoliated plants of smooth bromegrass and hybrid bromegrass, which could partially explain

the lower LAI in later growth. Development of LAI for the three bromegrasses was more rapid

following defoliation at the vegetative compared to stem elongation stage. Following defoliation

at the vegetative stage, rapid leaf development typically occurs from leaf intercalary meristems

(Langer 1972) and leaf primordia, which remain in the basal position of the tiller (Hyder1972). After defoliation at the stem elongation stage, new tillers from axillary buds become the most important source for canopy leaf area development (Olson and Richards 1988b; Richards et al. 1988), but axillary bud development is slower than growth of intercalary meristems.

In summary, the hypothesis that meadow bromegrass has greater above-and below-ground regrowth biomass than the other two species is rejected. Meadow bromegrass, however, did produce greater above-ground biomass than smooth bromegrass when defoliated at the stem elongation stage and more below-ground biomass than smooth bromegrass following all stages of defoliations. Above-and below-ground biomass of hybrid bromegrass was similar to meadow bromegrass. The hypothesis that LAI development was more rapid in meadow bromegrass than the other two bromegrasses is accepted.

4.5 Grazing management implications

In Saskatchewan, initial grazing of smooth bromegrass is recommended after mid-May (Harrison and Romo 1994). On the basis of the present study, all three bromegrasses can be grazed before mid-May in a year of above-average precipitation and average GDD, but at least 30-46 day rest period is required for leaf area reestablishment before a second grazing. If grazing is initiated after stem elongation, normally in June, meadow and hybrid bromegrass can produce more regrowth than smooth bromegrass. The length of the rest period for grazing after stem elongation should be more than that after the vegetative stage to establish a similar leaf area. Smooth bromegrass is relatively slower in developing leaf area after defoliation. Therefore, a higher defoliation height is recommended to provide resources to re-establish leaf area. Root development of the three species is negatively affected by grazing at any time of growth.

5.0 Tiller Density and Axillary Bud Development of Three Bromegrass (*Bromus*) Species in Response to Defoliation

Abstract Three bromegrass (*Bromus*) species used in western Canada have variable capacity to regrow following defoliation. Tiller development following defoliation is considered a key factor for regrowth. The objective of this study was to determine tiller and axillary bud number of meadow bromegrass (*Bromus riparius* Rehm.), smooth bromegrass (*Bromus inermis* Leyss.), and hybrid bromegrass (*B. riparius* X *B. inermis*) following defoliation at the vegetative and stem elongation stages in field and greenhouse environments. The field study was conducted in 2006 and 2007 in Saskatoon, Saskatchewan ($52^007'$ N, $106^038'$ W). Sods of the three species were removed from the field in early November of each year for the greenhouse study. Plants were clipped to 5cm height at the vegetative and stem elongation stages; an undefoliated control was also included. In the field study, tiller density was greatest in meadow bromegrass (2107, 1320 tillers m^{-2}), intermediate in hybrid bromegrass (1547, 840 tillers m^{-2}) and least in smooth bromegrass (1093, 520 tillers m^{-2}) following defoliation at the vegetative and stem elongation stages, respectively. In the undefoliated control, 15% fewer tillers of meadow bromegrass reached the reproductive stage compared to the other two bromegrasses. In the greenhouse, tiller densities were similar among species after defoliation. Regardless of growth environment, final tiller density after defoliation at the vegetative stage was similar to the undefoliated control, whereas tiller density was reduced by 35% following defoliation at the stem elongation stage. In the undefoliated control, total buds tiller^{-1} and elongated buds tiller^{-1} were greater in meadow

(7.1 and 1.8 buds tiller^{-1}) and hybrid bromegrass (7.2 and 1.6 buds tiller^{-1}) than smooth

bromegrass (6 and 1.1 buds tiller^{-1}), but no species differences were detected among species

following defoliation. Buds on regrowing tillers were visually smaller following defoliation at

the stem elongation stage compared to the undefoliated control. The key factors underlying the

rapid regrowth of meadow bromegrass were that a greater production of its tillers remained

vegetative and it had a greater density of tillers than the other two bromegrasses.

5.1 Introduction

Bromegrasses are widely cultivated in western Canada. Meadow bromegrass

(*Bromus riparius* Rehm.) is mainly used for pasture, and smooth bromegrass (*Bromus inermis*

Leyss.) is generally used for hay (Knowles et al. 1993). The hybrid bromegrass (*B. riparius* X *B.*

inermis) cultivar Knowles was developed by hybridizing smooth and meadow bromegrass

(Coulman 2004), and it has potential for both hay and pasture use. The three species have

variable regrowth potential following defoliation (Knowles et al. 1993; Coulman 2004). Tiller

development following defoliation may be important in differences in regrowth because the

growth of grasses depends on the size and number of tillers (Laude et al. 1968).

Tiller development following defoliation is unique for each grass species.

Bromegrasses vary in tillering after defoliation at the reproductive stage (Van Esbroeck et al.

1995). Caldwell et al. (1981) and Richards and Caldwell (1985) reported that wheatgrasses

(*Agropyron*) with similar phenology and physiological traits differed markedly in their ability to

produce new tillers following defoliation. Tillering of grasses is regulated by a number of factors

such as hormones, nutritional competition, and photosensitivity to the red to far-red light ratio

(Cline 1991; Murphy and Briske 1992; Tomlinson and O'Connor 2004). The apical portion of

the shoot, which includes the apical meristem and young leaves, exerts hormonal or nutritional

control on axillary bud growth following bud formation (Gregory and Veale 1957; Cline 1991). This control is always released after removing the apex (Paulsen and Smith 1969; Olson and Richards 1988b).

The developmental stage at the time of defoliation can affect tiller development in grasses because it is related to removal of apical meristems and activity of axillary buds (Branson 1953; Jewiss 1972; Paulsen and Smith 1969). If tillers are defoliated before internode elongation, tiller development is dependent upon regrowth from existing tillers and axillary buds (Jewiss 1972; Paulsen and Smith 1968); however, the majority of tillers develop from axillary buds following defoliation at the stem elongation stage in most cool-season grasses (Hyder 1972; Jewiss 1972). In addition, numerous environmental variables exert influences on tiller initiation after defoliation (Langer 1972). High temperatures inhibit tillering because of high respiration rates and lower soluble carbohydrate availability in plants (Langer 1972). Tiller density of cool-season grasses increases with light intensity (Langer 1972; Ashmun and Pitelka 1984), availability of soil moisture (Cook et al.1958) and soil nutrients (Langer 1972; Shaver et al. 1986). Thus, tiller development may vary under different environments.

A series of experiments were conducted with meadow, smooth, and hybrid bromegrasses under field and greenhouse environments to determine: 1) tiller development of the three bromegrasses following defoliation at two developmental stages; 2) the number of axillary buds, and; 3) the percentage of tillers reaching the reproductive stage in an undefoliated control. It was hypothesized that meadow bromegrass produces more axillary buds and tillers, and fewer reproductive tillers than smooth and hybrid bromegrasses following defoliation.

5.2 Materials and methods

5.2.1 Experimental design and treatments

5.2.1.1 Field experiment

The two variables examined in this experiment were three species (meadow, smooth, and hybrid bromegrass) and three defoliation treatments (defoliated at the vegetative stage; defoliated at stem elongation stage, and an undefoliated control). The experimental design was a split-plot with species arranged in the main plots, and defoliation treatments applied to sub-plots. Each treatment was replicated four times. Defoliation height was 5 cm above ground level.

5.2.1.2 Greenhouse experiment

Sods of the three bromegrass species were transferred to the greenhouse from the field in early November of 2006 and 2007 to assess of tiller density and axillary bud development. Sods were planted in 20cm pots using a soil mix that contains peat moss, medium grade vermiculite, Scott's "Osmocote Plus" fertilizer (16-8-12), and trace elements. Light was provided by high intensity sodium lamps with a day length of 16h at 21°C and a night period of 8h at 16°C. Sods were watered periodically when the soil surface became dry.

The experiment was a 3 X 4 factorial arrangement in a randomized-complete-block-design with treatment combinations of three bromegrass species (meadow, smooth and hybrid bromegrass) and four defoliation treatments (single defoliation at the vegetative stage; single defoliation at the stem elongation stage; defoliated at the vegetative stage and again after two weeks; and an undefoliated control). Each treatment was replicated four times. Defoliation height was 5cm above ground level.

5.2.2 Data collection

5.2.2.1 Tiller densities

The number of tillers in the field was recorded in two 15 x 20cm quadrats randomly placed over rows in each plot before all tillers were defoliated at the vegetative (first leaf collared) and stem elongation stages (second node visible) (Moore and Moser 1995). In each year, tiller numbers were recorded again after a similar number of GDD had accumulated following each defoliation (Table 5.1). Undefoliated controls were included in all plots. In the greenhouse, the number of initial tillers was counted in each pot before the experiment began and again after 60 days of growth (1260 GDD).

Two 15 x 20cm areas were randomly sampled from each field plot in undefoliated stands of the three bromegrass species on 23 June 2006 and 15 June 2007. Reproductive and non-reproductive tillers were counted, and the percentage of reproductive tillers was calculated. In the greenhouse, reproductive and non-reproductive tillers were counted for undefoliated plants.

Table 5.1. Dates of defoliation and measurement of tiller density of three bromegrasses defoliated at two stages of growth, and the corresponding growing-degree-days (GDD) between defoliation and measurement in a field study conducted in 2006 and 2007 at Saskatoon, Saskatchewan.

	Date of defoliation		Date of measurement		GDD (°C)	
	2006	2007	2006	2007	2006	2007
Vegetative stage						
all three bromegrasses	10 May	9 May	1 Aug	13 Jul	1465	964
Stem elongation stage						
meadow bromegrass	19 May	25 May	28 Jul	24 Jul	1271	1049
smooth bromegrass	22 May	31 May	1 Aug	27 Jul	1304	1045
hybrid bromegrass	22 May	26 May	1 Aug	24 Jul	1304	1041

5.2.2.2 Axillary buds

After counting the final numbers of tillers in the greenhouse, stem bases of each species were removed and soaked in water before washing with water under high pressure. Twenty tillers were randomly selected from the washed samples for each treatment. The existing and elongating numbers of axillary buds tiller $^{-1}$ were counted under microscope. Photographs of axillary buds were taken under a 2x power microscope.

5.2.3 Statistical analysis

Analysis of variance (ANOVA) was used to determine the effects of species and defoliation treatments on tiller density, axillary bud number, and the percentage of reproductive tillers. In the field study, data were analyzed as a split plot arrangement in a RCBD using SAS.9.1.3 Proc Mixed Model (SAS Institute Inc. 2003). Species, developmental stage of defoliation and their interactions were considered fixed effects and year, block, and block x species were considered as random effects in the model. The two sub-samples of tiller densities were averaged within each plot and means were used in the analysis. For the greenhouse study, data were analyzed as a two-way factorial arrangement using SAS 9.1.3 Proc Mixed Model (SAS Institute Inc. 2003). Species, developmental stage of defoliation, and their interaction were considered as fixed effects and block (replication), and year (repetition) were considered as random effects in the model. The final tiller density was adjusted using Analysis of Covariance to eliminate the effects of initial tiller differences. Data were further analyzed within each factor by performing Proc Mixed as a one-way ANOVA. The means were separated using least square means comparisons. The percentage of reproductive tillers was transformed using arcsine-

square-root transformation prior to statistical analysis, and then back-transformed for presentation.

5.3 Results

5.3.1 Tiller density

5.3.1.1 Field experiment

For the field study, species (P<0.01) and stage of defoliation (P=0.02) effects on tiller density were significant, but the interaction of these factors was not significant (P=0.13). Overall, tiller density was the greatest for meadow bromegrass, intermediate for hybrid bromegrass and least for smooth bromegrass (Table 5.2). Defoliation at the stem elongation stage reduced tiller density an average of 35%, but defoliation at the vegetative stage had no effect on tiller density compared to the undefoliated control.

In the undefoliated control, final tiller densities of meadow, smooth, and hybrid bromegrasses increased by 79, 29 and 59%, respectively, from the initial counts (Table 5.2). Similarly, final tiller densities of the three bromegrasses increased from the initial counts following defoliation at the vegetative stage with the highest percent increase (95%) in meadow bromegrass compared to 14% in smooth and 57% in hybrid bromegrass. The final tiller density was reduced in all species following defoliation at the stem elongation stage with the lowest decrease in meadow bromegrass (-15%) and the greatest decrease in smooth bromegrass (-51%).

Table 5.2. Tiller density of three bromegrass species after two months of growth in the field for undefoliated control or following defoliation to 5 cm at two stages of growth in 2006 and 2007 at Saskatoon, Saskatchewan.

Species	Undefoliated control			Defoliated at vegetative stage			Defoliated at stem elongation		
	-------------------------------- Tillers m^{-2}-------------------------------------
	Initial	Final	%[‡]	Initial	Final	%	Initial	Final	%
meadow bromegrass	1120a	2000 a^{z}	+ 79	1080a	2107a	+ 95	1547a	1320a	- 15
smooth bromegrass	933 b	1200 c	+ 29	960a	1093c	+ 14	1053c	520c	- 51
hybrid bromegrass	1013ab	1520 b	+ 50	987a	1547b	+ 57	1307b	840b	- 36
P	<0.01	<0.01		0.42	<0.01		<0.01	<0.01	
SEM[y]	193	222		213	74		212	57	

z Means within a column with the same lower case letter (*a-c*) are not significantly different (P≤0.05).
[y] Standard error of the mean.
[‡] Percentage of tiller increase (positive value) or decrease (negative value) compared to initial tiller count.

5.3.1.2 Greenhouse experiment

Stage of defoliation had a significant (P=0.05) effect on tiller density, but species (P=0.18) and the interaction of species by stage of defoliation (P=0.82) had no significant effect on tiller density. Overall, tiller density was reduced 39 and 42%, respectively, following defoliation at the stem elongation stage or defoliated two times at the vegetative stage compared to the undefoliated treatment; tiller densities were not affected by defoliation at the vegetative stage (Table 5.3).

Tiller density was compared among the species in each defoliation treatment (Table 5.3). The initial number of tillers had a significant effect on the final tiller count, and this was adjusted using analysis of covariance. The final tiller density was not significantly different among the three species within the same defoliation treatment. Similar to tiller development in the field, final tiller numbers of meadow, smooth, and hybrid bromegrass increased from initial counts in the undefoliated control and after defoliation at the vegetative stage. The final tiller

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density was reduced in all three species following defoliation at the stem elongation stage and

after being defoliated two times. In contrast to the responses in the field, the percentage increase

in tiller density was greater for smooth bromegrass after defoliation at the vegetative stage, but

the percent decrease in tiller density was less for smooth bromegrass after defoliation at the stem

elongation stage compared to the other two species.

Table 5.3. Tiller density of three bromegrass species after two months of growth in greenhouse in undefoliated control or following defoliation to 5cm height at different stages of growth. Data are from two trials conducted in 2006 and 2007.

Species	Undefoliated control			Defoliated at vegetative stage			Defoliated at stem elongation			Defoliated twice at vegetative stage		
	Initial	Final [y]	%[‡]	Initial	Final	%	Initial	Final	%	Initial	Final	%
meadow bromegrass	5696a	6500a	+32	5588a	6067a	+28	5542a	3833a	-20	5708a	3633a	-29
smooth bromegrass	2842c	5367a	+59	3054c	5500a	+42	3300b	3500a	- 8	3688c	3500a	-17
hybrid bromegrass	3925b	5800a	+44	4554b	5533a	+25	4088b	3467a	-15	4888b	3200a	-34
P	<0.01	0.12		<0.01	0.40		<0.01	0.54		0.01	0.43	
SEM	400	312		380	287		352	950		359	585	

Tillers m^{-2}

[y] Final tiller number in the table was adjusted by analysis of covariance.

z Means within a column with the same lower case letter (*a-c*) are not significantly different (P≤0.05).

[‡] Percentage of tiller increased (positive value) or decreased (negative value) compared to initial tiller count. The percentage was calculated using final tiller density before adjusting by analysis of covariance.

5.3.2 Axillary buds

Axillary buds are located in rows on opposite sides of the stem bases. These axillary

buds exhibited a size gradient along the stem; the uppermost buds were the largest and the basal

buds the smallest (Fig 5.4 and Fig 5.6). Most new tillers originated from buds located at the mid-

to upper positions of tiller bases (Fig 5.2 and Fig 5.10). Bud size did not differ visually among

three species. Bud size was visually smaller on regrowing tillers when defoliated at stem

elongation or after being defoliated two times (Fig 5.5 and Fig 5.6).

Total buds tiller^{-1} and elongated buds tiller^{-1} were significantly greater in meadow and hybrid bromegrass than smooth bromegrass in the undefoliated control (Table 5.4); however, species did not differ for these traits following defoliation.

Table 5.4. Total number of axillary buds and elongating tillers of three bromegrass species after two months of growth in greenhouse in undefoliated control or following defoliation to 5cm height at different stages of growth. Data are from two trials conducted in 2006 and 2007.

	Defoliation treatment							
Species	Undefoliated control		Defoliated at vegetative stage		Defoliated at elongation stage		Defoliated twice at vegetative stage	
	---buds tiller $^{-1}$---							
	Total	Elongated	Total	Elongated	Total	Elongated	Total	Elongated
meadow bromegrass	7.1 a^z	1.8 a	6.9 a	1.6 a	5.5 a	0.5 a	6.0 a	0.5 a
smooth bromegrass	6.0 b	1.1 b	6.3 a	1.3 a	5.8 a	0.6 a	5.5 a	0.6 a
hybrid bromegrass	7.2 a	1.6 a	6.7 a	1.2 a	5.3 a	0.5 a	6.3 a	0.3 a
P	0.01	0.03	0.30	0.16	0.46	0.62	0.14	0.15
SEM^y	0.7	0.2	0.6	0.2	0.4	0.3	0.5	0.3

^{z} Means within a column with the same lower case letter (*a-b*) are not significantly different (P≤0.05).
^{y} Standard error of the mean.
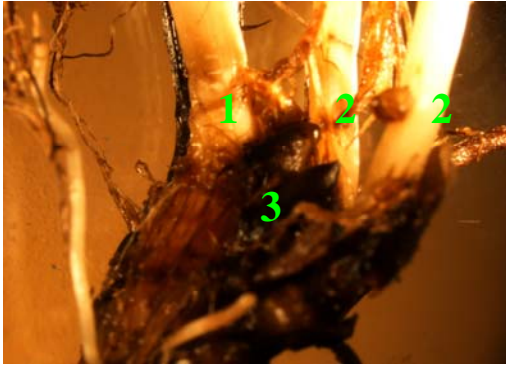
Figure 5.1 Axillary bud development of meadow bromegrass in undefoliated plant after two months of growth in the greenhouse (1=parent tiller；2= elongating bud；3= visible bud). *Photo amplified two times.

Figure 5.2 Axillary bud development of meadow bromegrass after two months of regrowth in the greenhouse following defoliation at the vegetative stage (1=parent tiller；2= elongating bud；3= visible bud).

Figure 5.3 Axillary bud development of meadow bromegrass after two months of regrowth in the greenhouse following defoliation at the stem elongation stage.
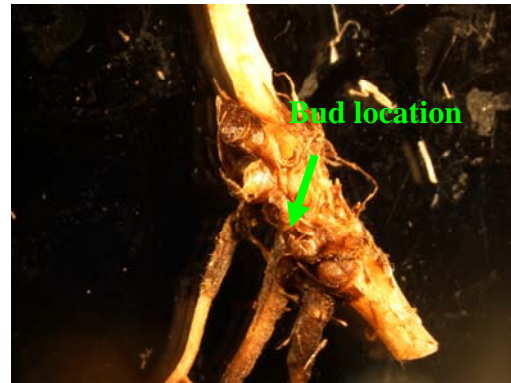
Figure 5.4 Axillary bud development of meadow bromegrass after two months of regrowth in the greenhouse following defoliation at the vegetative stage and an additional defoliation two weeks later.
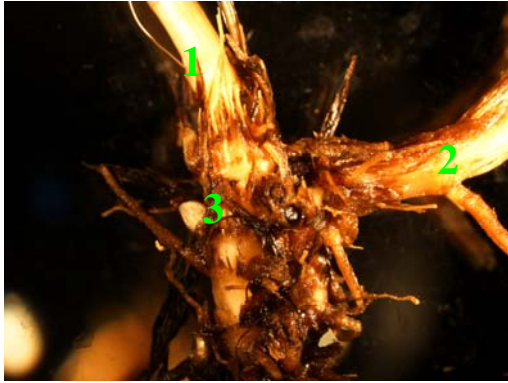
Figure 5.5 Axillary bud development of smooth bromegrass in undefoliated plant after two months of growth in the greenhouse (1=parent tiller；2= grown out bud；3= visible bud). *Photo amplified two times.

Figure 5.6 Axillary bud development of smooth bromegrass after two months of regrowth in the greenhouse following defoliation at the vegetative stage (1=parent tiller；3= visible bud).

Figure 5.7 Axillary bud development of smooth bromegrass after two months of regrowth in the greenhouse following defoliation at the stem elongation stage.
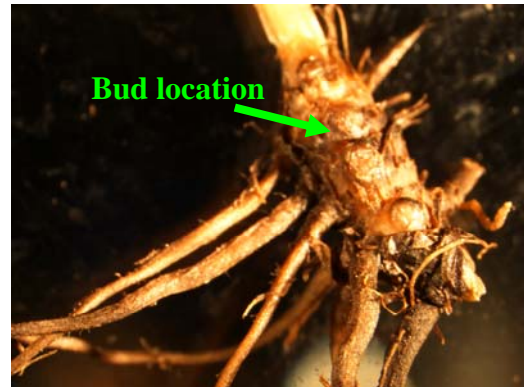
Figure 5.8 Axillary bud development of smooth bromegrass after two months of regrowth in the greenhouse following defoliation at the vegetative stage and an additional defoliation two weeks later.
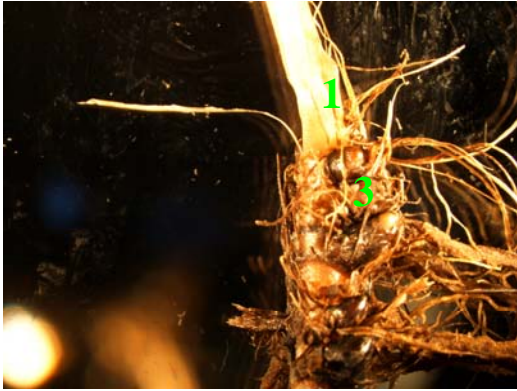
Figure 5.9 Axillary bud development of hybrid bromegrass in undefoliated plant after two months of growth in the greenhouse (1=parent tiller；3= visible bud). *Photo amplified two times.
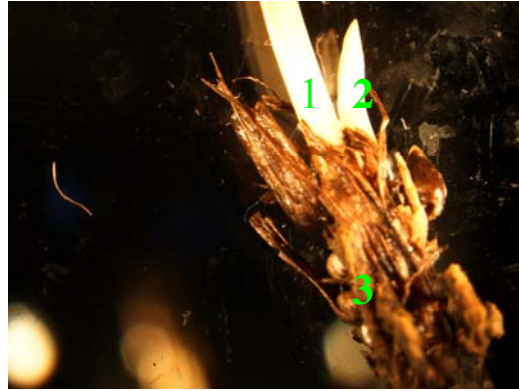
Figure 5.10 Axillary bud development of hybrid bromegrass after two months regrowth in the greenhouse following defoliation at the vegetative stage (1=parent tiller；2= elongating bud；3= visible bud).
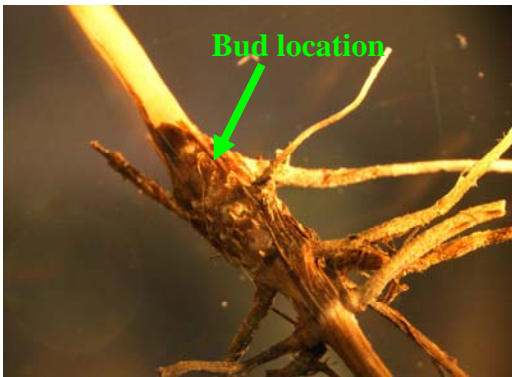
Figure 5.11 Axillary bud development of hybrid bromegrass after two months of regrowth in the greenhouse following defoliation at the stem elongation stage.
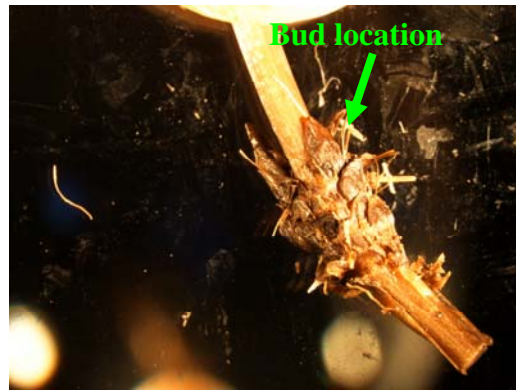
Figure 5.12 Axillary bud development of hybrid bromegrass after two months of regrowth in the greenhouse following defoliation at the vegetative stage and an additional defoliation two weeks later.

5.3.3 Reproductive tiller development for undefoliated control plants

Percentages of tillers reaching the reproductive stage significantly differed among species (P<0.01) in the field. A lower percentage of tillers reached the reproductive stage in meadow bromegrass (64%) than smooth (82%) and hybrid bromegrass (77%). In the greenhouse, a lower percentage of tillers reached the reproductive stage compared to the field, and the percentages were similar among the three bromegrass species (P=0.39).

Table 5.5. Percentages of tillers (%) producing inflorescences in undefoliated swards of meadow, smooth, and hybrid bromegrasses in the field and greenhouse in Saskatoon, Saskatchewan.

	Growth environment	
	Field	Greenhouse
meadow bromegrass	64 b[z]	50 a
smooth bromegrass	82 a	57 a
hybrid bromegrass	77 a	54 a
P	<0.01	0.40
SEM[y]	0.3	0.7

[z] Means within a column with the same lower case letter (*a-b*) are not significantly different (P≤0.05).
[y] Standard error of the mean.
[‡] Data are from two experiments conducted in 2006 and 2007.

5.4 Discussion

In the present study, tiller density was the highest for meadow bromegrass, intermediate for hybrid bromegrass, and lowest for smooth bromegrass following defoliation at the vegetative and stem elongation stages in the field. Van Esbroeck et al. (1995) reported higher tiller densities in meadow bromegrass and an experimental hybrid bromegrass population than smooth bromegrass after defoliation in the fall. When undefoliated, meadow bromegrass also had higher tiller density than smooth bromegrass (Ferdinandez and Coulman 2000). The difference in

tiller densities may be a key factor causing variation of regrowth among these three bromegrasses. Tiller development, however, is a complex process under the control of abiotic and biotic factors (Langer 1972). The abiotic environment was similar for the three bromegrasses in this study; thus, genetic or hormonal control likely played a role in tiller development for these three bromegrasses.

Existing tillers can continue to grow if defoliated before internode elongation because apical meristems are not removed (Jewiss 1972). Elevation of apical meristems during stem elongation makes grasses increasingly vulnerable to defoliation (Brown 1982), and loss of the apical meristem after defoliation causes tiller senescence in grasses (Davies 1976). In the present study, final tiller density increased from the initial count following defoliation at the vegetative stage, likely because of continued growth of defoliated tillers. The percentage increase in smooth bromegrass in the field study (14%) was much lower than hybrid (57%) and meadow bromegrass (95%). This suggests that fewer new tillers were initiated in smooth bromegrass; however, this could not be verified as axillary bud formation and elongation were not measured on field-grown plants. Following defoliation at the stem elongation stage, final tiller density was reduced in the three bromegrasses because defoliation caused senescence of elongated tillers. The reduction in density in the field study was greatest in smooth bromegrass, likely because of its higher percentage of elongated tillers. Early regrowth after defoliation at more mature growth stages depends on the size and the number of unelongated tillers (Jewiss and Powell 1966).

In contrast to the field study, final tiller densities following defoliation did not differ among the three bromegrasses in the greenhouse. Tiller development in the greenhouse was likely affected by plant preparation (see Appendix B) and growing conditions that were different from the field. Cutting done to prepare plants caused slower tiller growth or even senescence of

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some tillers in meadow bromegrass, but this was not observed in smooth bromegrass (see Appendix B). In addition, the initial number of tillers in meadow bromegrass was greater than smooth bromegrass, and growth may have been restricted by limited nutrients and lower light intensity in the greenhouse. Resource competition affects tillering, and tiller development is stimulated by high nutrient concentration (Langer 1972; Shaver et al. 1986; McIntyre 2001) and high light intensity (Langer 1972; Ashmun and Pitelka 1984). Hyder (1972) suggested that rhizomes of grasses always terminate in the development of tillers after defoliation. Smooth bromegrass produces rhizomes, whereas meadow bromegrass and hybrid bromegrass have a more caespitose growth form with short rhizomes. Consequently, rhizomes of smooth bromegrass could produce more tillers after defoliation, but rhizome spread was limited by pot wall in the greenhouse. This may have increased the numbers of tillers of smooth bromegrass per unit area in the pot. Some or all of these factors may have delayed or slowed tiller development in meadow bromegrass compared to smooth bromegrass in the greenhouse. In the present study, the greenhouse was used to provide a greater control of temperature and soil moisture than could be achieved in the field. Variation introduced during plant material preparation (see Appendix B) suggests that it was inappropriate to conduct this type of study in the greenhouse.

In the greenhouse study, increases in tiller numbers were not as variable among the species as in the field, likely because the total and elongated axillary buds did not vary among the three species. In a previous study, buds tiller^{-1} of two wheatgrass species with different tiller development were also similar after defoliation (Mueller and Richards 1986). Non-elongated buds remain viable throughout the growing season in wheatgrass species (Mueller and Richards 1986), and longevity of buds often exceeds that of their parent tillers (Hendrickson and Briske 1997). Elongation of basal buds into tillers depends on interactions of overall plant vigor,

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development stage, soil nutrients, carbohydrate reserves, and environmental conditions in addition to hormonal regulation (Paulsen and Smith 1969; Langer 1972; Ashmun and Pitelka 1984; Shaver et al. 1986; Murphy and Briske 1992).

In the present study, defoliation at stem elongation or defoliation two times during vegetative growth visually reduced bud size compared to undefoliated tillers. Tiller initiation from basal buds was also suppressed by defoliation in other grasses (Branson 1956; Ellison 1960; Jameson 1963). Bud growth was restricted by carbohydrate availability (Fletcher and Dale 1974; Blake and Tchapinski 1986). Carbohydrate concentrations are lower in bromegrass at the early stem elongation stage (Paulsen and Smith 1969), and carbon assimilation is also less after defoliation at this stage because of slower leaf area development (see Chapter 4), all of which may reduce bud size after defoliation at the stem elongation stage.

In summary, meadow bromegrass had a higher tiller density with a lower percentage of tillers reaching the reproductive stage compared to the other two species. In greenhouse studies, buds tiller $^{-1}$ and elongated buds tiller^{-1} were similar among the species. Therefore, the hypothesis that meadow bromegrass has higher tiller density was accepted, but that it has higher buds tiller $^{-1}$ was rejected. A greater percentage of tillers remaining vegetative was a key factor for more rapid tiller development in meadow bromegrass following defoliation at the stem elongation stage.

5.5 Grazing management implication

Meadow bromegrass produces more rapid regrowth than the other two species because of its higher tiller density and greater percentage of unelongated tillers. Consequently, this species should be the bromegrass of choice for grazing. Hybrid bromegrass is intermediate for these traits; therefore, it would be better for grazing than smooth bromegrass. Grazing at the

vegetative stage before internode elongation does not effect tiller density of the bromegrasses, and regrowth following defoliation at this stage is relatively rapid. Tiller densities of the bromegrasses decrease if grazed after stem elongation.

6.0 A Comparative Study of Etiolated Growth and Stem Base N Concentration of Three Bromegrass (*Bromus*) Species after Defoliation at Different Developmental Stages

Abstract Bromegrass species used in western Canada have a variable capacity to regrow following defoliation. Remobilization of organic reserves after defoliation may be important for regrowth of these grasses. A field study was conducted in 2006 and 2007 in Saskatoon, Saskatchewan to determine etiolated regrowth, N concentration in stem bases, and regrowth of meadow bromegrass (*Bromus riparius* Rehm.), smooth bromegrass (*Bromus inermis* Leyss.) and hybrid bromegrass (*B. riparius* X *B. inermis*) after defoliation to ground level at the vegetative, stem elongation, and reproductive stages. End-of-season etiolated growth was also determined. Etiolated regrowth of meadow bromegrass (56, 31 g m^{-2}) and hybrid bromegrass (59, 12 g m^{-2}) in the field was 29 and 84% greater than smooth bromegrass (41, 3 g m^{-2}) 10 days after defoliation at the vegetative and stem elongation stages, respectively. Meadow bromegrass produced 31% more etiolated growth than hybrid bromegrass following defoliation at the stem elongation stage. Meadow and hybrid bromegrass produced 54% more etiolated growth than smooth bromegrass at the end of season. Etiolated regrowth was similar among species when defoliated at the reproductive stage, averaging 62 g m^{-2}. When defoliated at more advanced developmental stages, the regrowth of these three species was more dependent on stored organic reserves with the dependence of smooth bromegrass on the reserves greater than the other two species. Nitrogen concentration in the stem base decreased with the advancing maturity, but it was similar among species. Meadow and hybrid bromegrass more rapidly utilized organic reserves to produce

regrowth than smooth bromegrass. If meadow and hybrid bromegrass are grazed, they can more rapidly re-establish shoot tissue than smooth bromegrass using stored reserves.

6.1 Introduction

Defoliation of plants removes leaf area, disrupting the photosynthetic capacity of plant. The energy source for regrowth of grasses immediately following defoliation is carbohydrate reserves in the stem bases (Reynolds and Smith 1962; Smith 1969). These reserves are predominantly non-structural carbohydrates, but also include nitrogenous compounds (White 1973).

The importance of N reserves for plant regrowth has been controversial for years. Stored nitrogenous compounds are considered important for regrowth in perennial ryegrass (*Lolium perenne* L.) (Ourry et al. 1988, 1989, 1990) because they are used in respiration, but these reserves are not alternately stored and utilized like carbohydrate reserves (White 1973). Some studies suggested that N required for regrowth of wheatgrasses (*Agropyron*) and cocksfoot (*Dactylis glomerata* L.) was supplied by uptake from the soil rather than stored reserves (Caldwell et al. 1981; Turner et al. 2006).

Even though the reserves are essential to plant vigor and recovery after defoliation (Busso et al. 1990), differences in regrowth among species are not related to the total carbon reserve or non-structural carbohydrate concentrations in the stem bases (Jameson 1963; Richards and Caldwell 1985). Similarly, differences in regrowth of smooth bromegrass cultivars could not be explained by the concentrations of carbohydrates (Paulsen and Smith 1969). Methodological errors in quantification of carbohydrate reserves or the possible contribution from other compounds such as nitrogenous constituents (Richards and Caldwell 1985) may affect the correlation between the quantity of carbohydrate reserves and regrowth.

The ability of a plant to rapidly remobilize stored reserves for synthesis of new above-ground tissues may also be an important indicator of regrowth potential. The etiolated growth technique (McKendrick and Sharp 1970) can be used to estimate the capacity to mobilize stored reserves for new above-ground tissues in the absence of photosynthesis (Richards and Caldwell 1985). The hypothesis tested in the present study is that meadow bromegrass more rapidly uses organic reserves to produce etiolated regrowth and this species has greater N concentration in the stem bases than hybrid and smooth bromegrass. The objectives of this study were to determine etiolated regrowth and N concentration in the stem bases of meadow, smooth, and hybrid bromegrasses at the vegetative, stem elongation, and reproductive stages, and also at the end of the growing season.

6.2 Data collection

6.2.1 Etiolated regrowth and regrowth in light

Etiolated regrowth was measured to estimate the amount of available organic reserves in plants and how rapidly these reserves were utilized. Regrowth in light was used to estimate photosynthetic assimilation and remobilization of organic reserve. The percentage of contribution from organic reserves to shoot growth was estimated by dividing etiolated regrowth by regrowth in light. Two 0.60m length of rows in each plot of the three bromegrasses were defoliated to ground level at the vegetative (first leaf collared), stem elongation (second node visible) and reproductive (inflorescence emerged) stages (Moore and Moser 1995). At each stage, one of the defoliated rows was covered by a light-excluding plastic box (41x27x18cm) to determine the etiolated regrowth in darkness. The inside walls of the boxes were covered with aluminum foil to ensure total darkness. A shovel was driven into the soil on the outside border of the box to a 20cm depth to cut rhizomes running to or from the soil area under the box. Etiolated

regrowth was determined by harvesting standing crop in a fixed 15 x 20cm central area of the

boxes 5, 10, 30, and 46d after defoliation. For defoliation at the reproductive stage, etiolated

growth was harvested 5, 10 and 30d after defoliation. The uncovered defoliated areas were used

to determine the amount of regrowth in light. All plants in the 15 x 20cm areas were clipped to

ground level after 46d to determine regrowth in light at each stage (30d after defoliation at the

reproductive stage). Within each defoliation treatment, there were similar numbers of

accumulated growing-degree-days (GDD) when the final cutting was taken (Table 6.1).

Harvested samples were dried in an oven at 60°C for 48h and weighed.

Table 6.1. Date of defoliation for regrowth of three bromegrasses and the corresponding total growing-degree-days (GDD) between defoliation and dry matter determinations in the field study in 2006 and 2007 at Saskatoon, Saskatchewan.

	Date of defoliation		Date of dry matter determination		GDD (°C)	
Developmental stage	2006	2007	2006	2007	2006	2007
Vegetative stage (46d[†])						
all three bromegrasses	8 May	12 May	23 Jun	27 Jun	676	632
Stem elongation stage (46d)						
meadow bromegrass	19 May	25 May	4 Jul	10 Jul	760	750
smooth bromegrass	22 May	31 May	7 Jul	16 Jul	789	791
hybrid bromegrass	22 May	26 May	7 Jul	11 Jul	789	756
Reproductive stage (30d)						
meadow bromegrass	12 Jun	16 Jun	13 Jul	17 Jul	608	564
smooth bromegrass	23 Jun	24 Jun	22 Jul	23 Jul	616	576
hybrid bromegrass	18 Jun	17 Jun	18 Jul	17 Jul	614	550

† Number of growth day following defoliation.

6.2.4.2 Etiolated regrowth at the end of the growing season

Etiolated regrowth was also determined for plant cores (4cm radius) collected in late

October 2006 and 2007 from the same bromegrass trials. This measurement was taken to

determine the amount of available organic reserves accumulated before winter. The plots were

clipped to 5cm in mid-August before taking soil cores. Twenty cores for each species were

collected in late October, cut to a uniform 12cm depth, and then placed in 12.5cm (diameter)

pots. Soil mix that contains peat moss, medium grade vermiculite, Scott's "Osmocote Plus" fertilizer (16-8-12), and trace elements was added to fill the pots. Pots were arranged in a growth chamber in a randomized-complete-block-design with four blocks. Sods were watered periodically when the soil surface became dry. Temperatures in the chamber were 21°C (16h) and 16°C (8h) without any light. Etiolated growth was harvested 5, 10, and 30d after placement in the growth chamber. No additional growth occurred after 30d; therefore, no further determinations of etiolated growth were made.

6.2.4.3 N concentration in stem bases

Twenty random samples of tillers were taken from each field plot at the vegetative, stem elongation, and reproductive, and also at seed maturity (Moore and Moser 1995) in 2006 and 2007. Stem bases (3cm in length) at each growth stage were gently washed with cold water to remove soil. The washed stem bases were placed in a paper bag, and dried at 60°C in a forced-air oven for 48h. The dried stem bases were bulked by species before grinding in a Wiley Mill through a 1-mm mesh screen and stored in airtight plastic bags. N concentration in the stem bases of the three species was determined using a Leco FP 428 Nitrogen Analyzer (Leco Corporation, USA).

6.2.5 Statistical analysis

Analysis of variance (ANOVA) (SAS Institute Inc. 2003) was used to compare etiolated growth and regrowth among species at each growth stage. Etiolated growth at the end of season was compared within each sampling date. End-of-season etiolated growth was also compared across sampling dates considering date as a fixed effect. When ANOVA indicated

significant differences among species (P≤0.05), the means were separated using least square means comparison. The percent N concentration was transformed using arcsine-square-root transformation prior to statistical analysis (Zar 1984), and data were analyzed using years as replications. Data were back-transformed for presentation.

6.3 Results

6.3.1 Etiolated regrowth in the field

Meadow and hybrid bromegrass produced similar amounts of etiolated regrowth following defoliation at the vegetative stage, averaging 33 and 57 g m^{-2} after 5 or 10 days, respectively (Table 6.2). Smooth bromegrass produced 31% less etiolated regrowth than meadow and hybrid bromegrass at the vegetative stage. When defoliation occurred at the stem elongation stage, cumulative etiolated regrowth was greatest in meadow bromegrass, intermediate in hybrid bromegrass and least in smooth bromegrass after 5 and 10 days (Table 6.2). By 30 days and thereafter, however, etiolated regrowth was similar among the three species for any defoliation at any growth stage. Cumulative etiolated regrowth did not differ among three species defoliated at the reproductive stage (Table 6.2).

Table 6.2. Cumulative etiolated regrowth of three bromegrasses after defoliation at different developmental stages in a field study conducted in 2006 and 2007 at Saskatoon, Saskatchewan.

	Days after defoliation			
Developmental stage	5	10	30	46
	-------------------------------- g m^{-2} ----------------------------			
Vegetative				
meadow bromegrass	31 a^Z	56 a	94 a	97 a
smooth bromegrass	22 b	41 b	82 a	92 a
hybrid bromegrass	35 a	59 a	98 a	102 a
P	0.01	0.03	0.22	0.47
SEM^y	7	19	39	40
Stem elongation				
meadow bromegrass	17 a	31 a	78 a	93 a
smooth bromegrass	1c	3 c	56 a	77 a
hybrid bromegrass	7 b	12 b	63 a	68 a
P	<0.01	<0.01	0.10	0.11
SEM	2	4	9	15
Reproductive				
meadow bromegrass	22 a	35 a	65 a	-
smooth bromegrass	21 a	32 a	60 a	-
hybrid bromegrass	22 a	30 a	62 a	-
P	0.99	0.61	0.86	
SEM	4	4	7	

^{z} Means within a column at each developmental stage with the same letter (*a-c*) were not significantly different (P≤0.05).
^{y} Standard error of the mean.

6.3.2 Etiolated regrowth at end of the growing season

The species x day interaction had no significant effect (P=0.24) on end-of-season etiolated regrowth, indicating species response was the same across sampling dates. Etiolated regrowth differed among species (P<0.01). Meadow and hybrid bromegrass had more cumulative etiolated regrowth than smooth bromegrass at all dates, but meadow and hybrid bromegrass did not differ (Table 6.3).

Table 6.3. Cumulative etiolated regrowth for field cores placed in pots in a growth chamber for three bromegrass species sampled at the end of October 2006 and 2007.

	Days after defoliation		
Species	5	10	30
	------------------------- g m^{-2} ----------------------		
meadow bromegrass	92 a^z	152 a	210 a
smooth bromegrass	36 b	62 b	96 b
hybrid bromegrass	90 a	140 a	190 a
P	<0.01	<0.01	<0.01
SEM^y	48	58	84

^{z} Means within a column with the same letter (*a-b*) were not significantly different (P≤0.05).
^{y} Standard error of the mean.

6.3.3 Regrowth in light

Regrowth dry matter in light did not vary significantly (P≥0.05) among the three bromegrass species after defoliation at the vegetative stage (Table 6.4). Cumulative etiolated regrowth was 12, 9, and 10 % of regrowth in light for meadow, smooth, and hybrid bromegrasses, respectively.

Meadow bromegrass and hybrid bromegrass produced significantly more dry matter in light than smooth bromegrass after defoliation at the stem elongation and reproductive stages (Table 6.5). Cumulative etiolated regrowth was 21, 44, and 33 % of the regrowth in light for meadow, smooth, and hybrid bromegrass, respectively, when defoliated at the stem elongation stage. When defoliated at the reproductive stage, cumulative etiolated regrowth was 19, 42, and 25% of that of regrowth in light for meadow, smooth, and hybrid bromegrasses, respectively.

Table 6.4. Etiolated regrowth and regrowth in light of three bromegrasses after 46days (30days for the reproductive stage) of growth in the field following defoliation to ground level at three stages in 2006 and 2007 at Saskatoon, Saskatchewan.

Developmental stage	meadow bromegrass	smooth bromegrass	hybrid bromegrass	P	SEM [y]
	---------------------------- $g\ m^{-2}$ ------------------------				
Vegetative					
regrowth in light	799 a	1003 a	1007 a	0.03	127
etiolated regrowth	97 (12%[‡])	92 (9%)	102 (10%)		
Stem elongation					
regrowth in light	633 a[z]	247 b	498 a	<0.01	148
etiolated regrowth	93 (15%)	77 (32%)	68 (14%)		
Reproductive					
regrowth in light	334 a	143 b	250 a	<0.01	51
etiolated regrowth	65 (19%)	59 (42%)	62 (25%)		

[z] Means within a row with the same lower case letter (*a-b*) are not significantly different (P≥0.05).
[y] Standard error of the mean.
[‡] Percentage of etiolated regrowth to total regrowth dry matter.

6.3.4 N concentration in stem bases

N concentration in the stem bases was similar among species (P=0.78), and the species x developmental stage interaction was not significant (P=0.97); however, N concentration in stem bases of the three bromegrasses was reduced significantly (P<0.01) with advancing plant maturity until the reproductive stage of growth (Fig. 6.3).
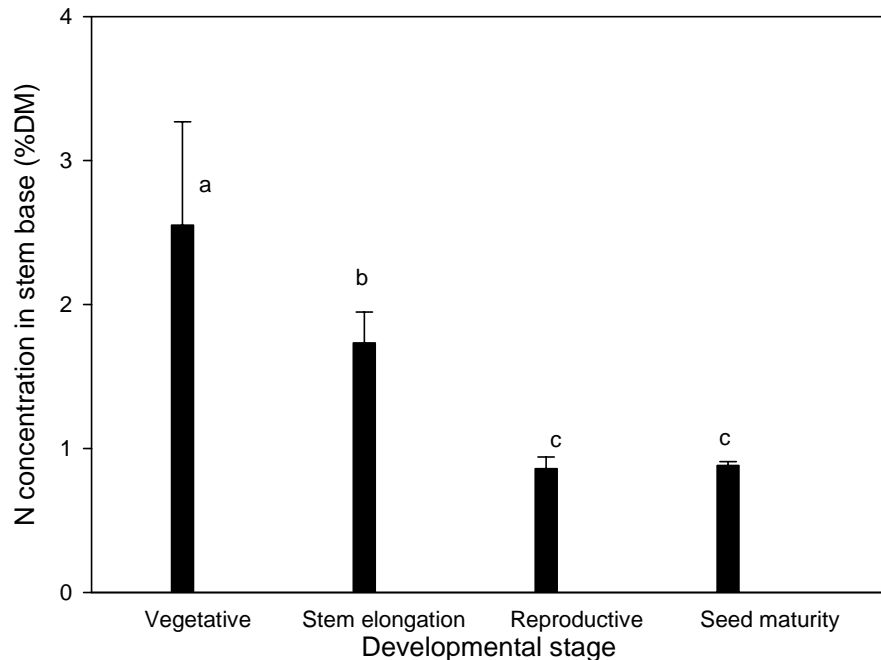
Figure 6.3 N concentrations in stem bases (% of dry matter) of three bromegrass species at different developmental stages in the field studies of 2006 and 2007 in Saskatoon, Saskatchewan. Bars are means + SE. Means with the same lower case letter (*a-c*) are not significantly different (P≤0.05).

6.4 Discussion

Etiolated regrowth varied among the three bromegrasses for up to 10 days after defoliation at the vegetative and stem elongation stages, and up to 30 days at the end of season. Meadow and hybrid bromegrass had greater etiolated regrowth than smooth bromegrass. In a previous study, etiolated growth in early spring was also greater in meadow bromegrass than smooth bromegrass for up to 30 days (Lardner et al. 2003). More etiolated regrowth for meadow and hybrid bromegrasses in the early growth suggests that these species were able to more rapidly access reserves to synthesize new growth following defoliation. Faster development of above-ground tissue using stored reserves would allow these species to rapidly re-establish photosynthetic area following defoliation, and in turn reduce the dependence on stored reserves.

In addition, more cumulative etiolated regrowth of meadow and hybrid bromegrasses than smooth bromegrass at the end of the season suggests meadow and hybrid bromegrasses also accumulate more organic reserves before winter.

Fructosan is the primary carbohydrate in smooth bromegrass, and the fructosan concentration in the stem bases declined from the vegetative stage, was lowest at the stem elongation stage, and gradually reached its highest amount at the reproductive stage (Reynolds and Smith 1962; Paulsen and Smith 1968). Richards and Caldwell (1985) reported that meristematic limitations for the reallocation of stored resources appeared more important than the amount of stored or assimilated energy in wheatgrass species. Lack of meristems for regrowth, particularly in smooth and hybrid bromegrass, may explain why etiolated regrowth was initially poor at the stem elongation stage. The large increase in etiolated growth after 30 days suggests that carbohydrates may not have been limiting. Regrowth following defoliation at the reproductive stage occurred under high temperatures and decreasing rainfall 30 days after defoliation, which may explain why total etiolated regrowth was less than that at the vegetative stage.

Meadow and hybrid bromegrasses produced about twofold greater etiolated regrowth in the growth chamber than the field, while etiolated regrowth of smooth bromegrass was similar in the two studies. In many temperate grass species, the lowest amount of carbohydrates occurs after early spring growth initiation, and the maximum level is reached after seed-shattering in fall (Reynolds and Smith 1962; Menke and Trlica 1981). The differences in the two former species may indicate a much higher storage of reserves prior to winter than growing season. The same may be true for smooth bromegrass; however, this species develops a deep root system (Gist and Smith 1948) and may store organic material in the deeper root zones, which would not be

assessed in our study because roots were cut to 12 cm for the growth chamber study. The environmental conditions under which the etiolated growth occurred were quite different between the field and growth chamber. Consistent watering of plants in the growth chamber may have ensured a more complete use of water soluble carbohydrates for etiolated growth.

Contribution of stored carbon reserves to shoot regrowth and respiration was much lower than the contribution of photosynthetic assimilation in several cool-season grasses (Davidson and Milthorpe 1966; Richards and Caldwell 1985; Donaghy and Fulkerson 1997). In our study, cumulative etiolated regrowth was only 12, 9 and 10% of the regrowth in light for meadow, smooth and hybrid bromegrass, respectively, following defoliation at the vegetative stage after 46 days. This suggests that most of the regrowth at this growth stage was derived from photosynthesis. Cumulative etiolated growth was a higher percentage of regrowth in light after defoliation at the stem elongation and reproductive stages, with the percentage highest for smooth bromegrass (44 and 42%, respectively). When defoliated at these more advanced developmental stages, regrowth appears to be more dependent on stored organic reserves, likely because of a greater amount of photosynthetic area is removed after stems have elongated than when they are vegetative (see Chapter 4). Relative to the other two bromegrasses, smooth bromegrass relied more on organic reserves after defoliation at later developmental stages; however, the regrowth of smooth bromegrass was less.

In the present study, N concentration in the stem base declined from the vegetative to reproductive stages of growth, and the three bromegrass species had similar N concentrations in stem bases at all growth stages. N concentrations in the storage organs of smooth bromegrass were greatest in young plants and then declined until the plant headed (Paulsen and Smith 1969). Even though there is evidence that nitrogen is remobilized from stubble to growing leaves

following defoliation in cool-season grasses (Phillips et al. 1983; Ourry et al. 1988, 1990), N content is relatively small compared to stored carbohydrates reserves (White 1973). Once enough carbohydrate reserves are available for respiration, plants are able to take up the required N from soil. The amount of N required for regrowth in wheatgrass and cocksfoot was apparently supplied by soil uptake rather than stored reserves (Caldwell et al. 1981; Turner et al. 2006). In the present study, meadow bromegrass maintains relatively larger below-ground biomass than smooth bromegrass after defoliation (see Chapter 4), which indicated a larger root volume and surface area in meadow bromegrass. Plants with a large root system or high root proliferation rates can occupy greater soil volumes and gather a greater share of soil resources (Caldwell et al. 1987). Larger below-ground biomass of meadow bromegrass suggests a greater soil N uptake of meadow bromegrass.

In summary, the hypothesis that meadow bromegrass can more rapidly use stored reserves to produce etiolated growth than the other two bromegrasses is supported in our study. The hypothesis that N concentration in the stem bases of meadow bromegrass was the greater than the other two bromegrass species is rejected. Etiolated regrowth was the greatest in meadow bromegrass, intermediate in hybrid bromegrass and least in smooth bromegrass, but this difference occurred only in the initial phase of growth. N concentrations in the stem bases were similar among species at all stages of defoliation, and it is probably not a major factor affecting regrowth in these three bromegrass species.

6.5 Grazing management implication

Organic reserves in the stem bases of bromegrasses is important for recovery of growth after defoliation when photosynthetic production is inadequate. If meadow and hybrid bromegrass are grazed at the vegetative or stem elongation stages, they can more rapidly re-

establish shoot tissue than smooth bromegrass in the early phase of regrowth. Therefore, in intensive rotational grazing systems, meadow and hybrid bromegrass would be superior to smooth bromegrass because of more rapid initial regrowth.

7.0 Effect of Defoliation on Leaf Expansion and Net Photosynthesis of Three Bromegrass (*Bromus*) Species

Abstract   Achieving a positive carbon balance after defoliation of plants is necessary for further growth and development. Morphological and physiological traits related to carbon assimilation are important to achieve a positive carbon balance. The objective of this study was to determine expansion of leaves, leaf-to-stem ratios, and above-ground biomass of meadow bromegrass (*Bromus riparius* Rehm.), smooth bromegrass (*Bromus inermis* Leyss.) and hybrid bromegrass (*B. riparius* X *B. inermis*) after defoliation at various growth stages and to determine the leaf photosynthetic rates. Plants of the three bromegrass species were removed from the field and planted in 20cm pots in a greenhouse. Plants were clipped to a 5cm height at the vegetative and stem elongation stages, or they were defoliated at the vegetative stage and again after 14 days. Meadow bromegrass produced 18-22% more regrowth than smooth bromegrass following defoliation at different stages, and 17% more regrowth than hybrid bromegrass when defoliated at the stem elongation stage. The leaf-to-stem ratio in regrowth of meadow bromegrass was more than twofold greater than that of smooth bromegrass, and about 1.4 times greater than that of hybrid bromegrass. The leaf-to-stem ratio in hybrid bromegrass was about 1.7 times greater than smooth bromegrass. Smooth bromegrass, however, expanded individual leaf area 1.5 times faster than meadow bromegrass and hybrid bromegrass. Photosynthetic rates were not different among the three bromegrasses, averaging 3.29µmol m^{-2}s^{-1}. More rapid regrowth of meadow bromegrass compared to smooth bromegrass and hybrid bromegrass was not related to expansion of its

individual leaf area and photosynthetic rate. A higher leaf-to-stem ratio in meadow bromegrass is likely an advantage for rapid carbon assimilation following defoliation.

7.1 Introduction

Bromegrass species are widely cultivated in western Canada. Meadow bromegrass (*Bromus riparius* Rehm.) is mainly used for pasture, while smooth bromegrass (*Bromus inermis* Leyss.) is generally for hay production (Knowles et al. 1993). The hybrid bromegrass (*B. riparius* X *B. inermis*) cultivar Knowles, which has potential for hay and pasture, was developed by hybridizing smooth and meadow bromegrass (Coulman 2004). The three species have variable regrowth potential following defoliation (Knowles et al. 1993; Coulman 2004).

It has been well established that defoliation reduces carbohydrate reserves in grasses (Reynolds and Smith 1962; Davidson and Milthorpe 1966; White 1973; Trlica and Cook 1971, 1972). Rapid carbon assimilation following defoliation is necessary to achieve a positive carbon balance for further growth and development. Photosynthetic rate and factors contributing to leaf area development should be considered in assessing the photosynthetic capacity of plants. Meadow bromegrass has narrow, pubescent leaves (Knowles et al. 1993) whereas smooth bromegrass has broader, glabrous leaves (Vogel et al.1996). The leaf pubescence of the hybrid bromegrass more closely resembles that of meadow bromegrass (Ferdinandez and Coulman 2000). Pubescence on the leaf surface modifies energy balance of the leaves by reducing light penetration to chloroplasts (Liakopoulos et al. 2006), which in turn reduces photosynthetic rate because of decreased light absorption (Ehleringer et al. 1976). Moreover, leaves of meadow bromegrass have less protein than the other two bromegrasses (Ferdinandez and Coulman 2001). In C_3 species, RuBP carboxylase-oxygenase, an enzyme used in the Calvin cycle to catalyze the first major step of carbon fixation, represents approximately 20-60% of total soluble protein in

the leaves (Maurice et al. 1979). Thus, morphological and physiological differences in the leaves may cause differences in photosynthetic rates among the bromegrass species. In addition, other cool-season grasses have enhanced or a slower decline of photosynthetic rate in fully expanded leaves of undamaged tillers following a partial defoliation (Gifford and Marshall 1973; Detling and Painter 1983). Partial defoliation of bromegrass swards is common under grazing, and more rapid regrowth of meadow bromegrass may be associated with enhanced photosynthetic rate in the leaves of remaining tillers following partial defoliation.

Meadow bromegrass has greater LAI than smooth bromegrass during regrowth in late summer, but leaves tiller^{-1} and leaf area tiller^{-1} are greater in smooth bromegrass than meadow bromegrass, and an experimental hybrid bromegrass population is similar to smooth bromegrass (Van Esbroeck et al. 1995). Leaf appearance rate tiller^{-1} after grazing is also greater in smooth bromegrass than meadow bromegrass after grazing (Lardner et al. 2002). Expansion of individual leaf area may also contribute to total LAI development.

The hypotheses of this study were: 1) meadow bromegrass has lower photosynthetic rates than smooth and hybrid bromegrass in undefoliated conditions, but the photosynthetic rates of meadow bromegrass are greater following partial defoliation and; 2) meadow bromegrass has a greater rate of individual leaf area expansion than smooth and hybrid bromegrass. The objectives of this study were to determine: 1) leaf area expansion rate, leaf-to-stem ratios and above-ground biomass following defoliation at different stages of development, and 2) net photosynthesis of the three bromegrass species in an undefoliated control and after partial defoliation.

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7. 2 Materials and methods

7.2.1 Experimental design

Sods of the three bromegrass species were transferred from the field in early November of 2006 and 2007. Sods were planted in 20cm (diameter) pots using soil mix that contained peat moss, medium grade vermiculite, Scott's "Osmocote Plus" fertilizer (16-8-12) and trace elements. The experiment was a 3 X 4 factorial arrangement in a randomized-complete-block-design with treatment combinations of three bromegrass species and four defoliation treatments (single defoliation to 5cm at the vegetative stage; single defoliation to 5cm at the stem elongation stage; defoliation to 5cm at the vegetative stage and again two weeks later; and an undefoliated control). Each treatment was replicated four times. Sods were watered periodically when the soil surface became dry. Light was provided by high intensity sodium lamps with a day length of 16h at 21°C and night period of 8h at 16°C.

Additional sods of the three bromegrass were transferred from the field to the greenhouse in early spring of 2007 and 2008 to measure photosynthetic rate. Growth conditions and watering regime were identical to the above experiment. The experiment was a 2 x 3 factorial in a randomized-compete-block-design with treatment combinations of three bromegrass species and two defoliation regimes (defoliated at the vegetative stage to remove 80% of tillers and an undefoliated control). Each treatment was replicated four times.

7.2.2 Data collection

7.2.2.1 Leaf area expansion

At the 2-3 leaf stage, 20 individual tillers from each of the three species were randomly selected and marked with rings near the centre of pots of the undefoliated control and

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the single defoliation at vegetative stage. Newly emerging leaves (4th and 5th leaves) of the defoliated and undefoliated tillers were marked using a permanent marker on the first day, and again 5 days later, and expanded leaf area day $^{-1}$ (cm^2day^{-1}) was calculated.

7.2.2.2 Leaf-to-stem ratio and above-ground biomass

To determine above-ground biomass and calculate leaf-to-stem ratio, all plants were harvested after 60 days. Fifteen tillers were randomly selected from each pot for leaf-to-stem ratio determinations. All samples were placed in separate paper bags, dried in a forced air oven at 60°C for 48h, and then weighed. The leaf-to-stem ratio was calculated from dry weights of the leaves and stems (including the leaf sheath).

7.2.2.3 Net photosynthesis

Measurements of net photosynthesis were performed on 4 partially defoliated and 4 undefoliated plants from each of the three bromegrass species. Partial defoliation was done by clipping to 5cm at the 3-4 leaf stage to remove 80% of the tillers in each pot, leaving the remaining 20% of tillers undefoliated. Leaf gas exchange measurements were taken on the uppermost fully expanded leaf of two remaining undefoliated portions of tillers in the defoliated plants. Six gas exchange measurements were taken on the same leaf blade every other day for 6 days using a LI-6200 Photosynthesis Measurement System (LI-COR, Inc.). The same measurements of photosynthetic rates were taken on undefoliated plants.

Measurements were taken between 1130h and 1330h to attempt to minimize variation of light intensity. A leaf blade was held horizontally in the leaf chamber during the 30-second measurement period. The area of the leaf blade enclosed in the chamber was determined

at the end of the experiment, and photosynthetic rate was calculated. Leaf chamber CO_2

concentration was near 335 ppm, and leaf temperatures were 23-25°C during the measurements.

Relative humidity in the air ranged from 35-40%. The K test is an assurance test to verify that

the LI-6200 system is working properly; if so, the K value will range between 1-1.5. The K test

was conducted before each measurement, and values ranged from 1.19 to 1.35. Light intensity

was highly variable during the measurements (Table 7.1).

Table 7.1. Light intensity (μmol m^{-2}s^{-1}) during the six days of measurement of gas exchange rate.

	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Trial one	895	510	128	107	572	927
Trial two	244	167	269	254	253	155
Means	570	339	199	181	413	541

7.2.3 Statistical Analysis

Data from the two repetitions (year) of the experiment were analyzed as a two-way

factorial (three bromegrass species and four defoliation treatments) in a randomized-complete-

block-design. Analysis of variance (ANOVA) of expanded areas of fourth and fifth leaves,

above-ground biomass and the leaf-to-stem ratio of the three bromegrass species was conducted

using SAS 9.1.3 Proc Mixed Model (SAS Institute Inc. 2003). When ANOVA indicated

significant differences (P≤0.05), the means were separated using least square means comparison.

The two measurements of photosynthetic rate were averaged for each treatment

before data were averaged across the two experimental replications (year). Data were analyzed as

a repeated measurement in a randomized-complete-block-design using the Proc Mixed Model of

SAS (SAS Institute Inc. 2003). Bromegrass species, defoliation, and their interactions were

considered fixed effects, block was considered a random effect, and measurement day was

treated as a repeated measure in the model. When ANOVA indicated significant differences

(P≤0.05), the means were separated using least square means comparison.

7.3 Results

7.3.1 Leaf area expansion

Leaf area expansion of fourth and fifth leaves of individual tillers were significantly

different among species (both P<0.01), but leaf area expansion was not affected by defoliation

(P=0.89, P=0.23) or the species x defoliation interaction (P=0.78, P=0.71). Data were further

analyzed with a one-way ANOVA (Table 7.2). Smooth bromegrass increased the surface area of

fourth and fifth leaves 1.5 times faster than meadow or hybrid bromegrass (Table 7.2), but there

was no difference between meadow bromegrass and hybrid bromegrass.

Table 7.2. Leaf area expansion of fourth and fifth leaves of three bromegrass species at the vegetative stage.

	Leaf area expansion	
Bromegrass species	4th leaf lamina	5th leaf lamina
	---------------------------- $cm^2\ day^{-1}$ -------------------------	
meadow bromegrass	0.89 b[z]	0.89 b
smooth bromegrass	1.27 a	1.51 a
hybrid bromegrass	0.99 b	1.01 b
P	<0.01	<0.01
SEM[y]	0.17	0.09

[z] Means within a column with the same letter (*a-b*) are not significantly different (P≤0.05).
[y] Standard error of the mean.

7.3.2 Above-ground biomass

Above-ground biomass differed significantly among species (P<0.01) and defoliation

treatments (P<0.01), but the interaction of species x defoliation was not significant (P=0.37).

Above-ground biomass was further compared among species at each defoliation (Table 7.3).

With no defoliation, meadow bromegrass produced 23% greater above-ground biomass than hybrid bromegrass, but above-ground biomass was similar between meadow and smooth bromegrass, and between smooth bromegrass and hybrid bromegrass (Table 7.3). Meadow bromegrass produced 18-22% more regrowth than smooth bromegrass after all defoliations, but the regrowth of meadow bromegrass was not significantly different from hybrid bromegrass except after the stem elongation stage. Regrowth of smooth and hybrid bromegrass did not differ significantly among all defoliation treatments, averaging 590 g m^{-2}.

Table 7.3. Above-ground biomass of three bromegrass species after 60 days (1260GDD) growth in a greenhouse in an undefoliated control or following defoliation to 5cm at different developmental stages. Data are from two experiments conducted in 2006 and 2007.

Species	Undefoliated Control	Defoliated at vegetative stage	Defoliated at stem elongation	Defoliated at vegetative stage + two weeks later
	--- g m^{-2} ---			
meadow bromegrass	1239 a^z	937 a	663 a	517 a
smooth bromegrass	1127 ab	775 b	561 b	404 b
hybrid bromegrass	954 b	822 ab	551 b	425 ab
P	0.05	0.04	0.04	0.05
SEM ^{y}	79	85	86	99

^{z} Means within a column at each developmental stage with the same letter (*a-b*) are not significantly different (P≤0.05).
^{y} Standard error of the mean.

7.3.3 Leaf-to-stem ratio

The species (P<0.01) and defoliation (P<0.01), and the species and defoliation interactions (P<0.01) had significant effects on the leaf-to-stem ratio. Data were subjected to additional analysis by using a one-way ANOVA to examine the interaction effect of species and defoliation treatments (Table 7.4). When not defoliated, leaf-to-stem ratios did not differ among the three bromegrass species, averaging 0.72 (Table 7.4). The leaf-to-stem ratio of smooth bromegrass was at least 46% less than meadow bromegrass after defoliation, and at least 30% less than hybrid bromegrass after defoliation at the stem elongation and defoliated two times.

Meadow bromegrass also had a higher leaf-to-stem ratio than hybrid bromegrass at all

defoliation except when defoliated at the vegetative stage.

Table 7.4. Leaf-to-stem ratio of three bromegrass species after two months growth in a greenhouse in an undefoliated control or following defoliation to 5cm at different developmental stages. Data are from two experiments conducted in 2006 and 2007.

Treatment		
Defoliation	Species	Leaf-to-stem ratio
Undefoliated control		
	meadow bromegrass	0.87 def [z]
	smooth bromegrass	0.62 f
	hybrid bromegrass	0.68 ef
Single defoliation at the vegetative stage		
	meadow bromegrass	1.55 c
	smooth bromegrass	0.85 def
	hybrid bromegrass	1.17 cde
Single defoliation at the stem elongation stage		
	meadow bromegrass	2.82 a
	smooth bromegrass	1.19 cde
	hybrid bromegrass	2.22 b
Defoliated at the vegetative stage + two weeks later		
	meadow bromegrass	3.34 a
	smooth bromegrass	1.29 cd
	hybrid bromegrass	2.15 b
P		<0.01
SEM [y]		0.23

[z] Means within a column with the same letter (*a-f*) are not significantly different ($P \leq 0.05$).
[y] Standard error of the mean.

7.3.4 Net photosynthesis

Day of measurement had a significant ($P<0.01$) effect on net photosynthesis of

individual leaves, but species, defoliation, and the defoliation x species interaction did not have a

significant affect ($P \geq 0.05$) on net photosynthesis for the three bromegrass species. Differences in

net photosynthesis among the measurement days may be associated with variation in light

intensity. The three bromegrass species exhibited similar net photosynthesis, averaging 3.29µmol

$m^{-2}s^{-1}$ (Fig 7.1). In addition, net photosynthesis of individual leaves in partially defoliated and

undefoliated control plants was not significantly ($P \geq 0.05$) different.
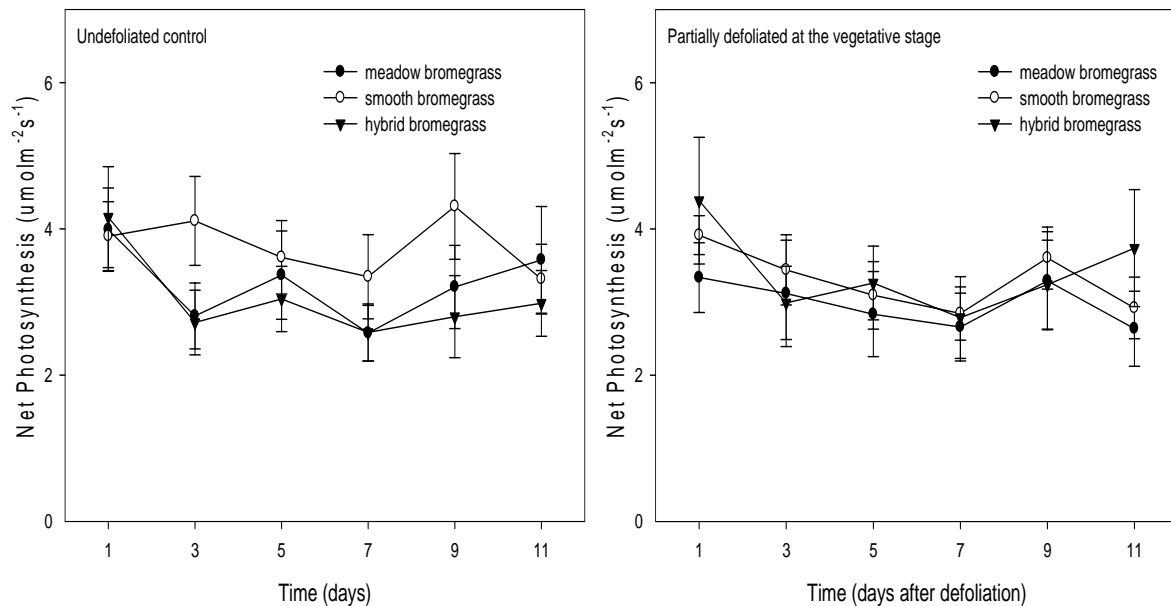
Figure 7.1. Net photosynthesis of three bromegrass species in undefoliated or after partial defoliation at the vegetative stage in a greenhouse. Data are means of two experimental replications and each point represents means of eight readings. Bars are means ± SE.

7.4 Discussion

Rapid leaf area establishment is a desirable trait for regrowth in grasses (Caldwell et al. 1981; Briske 1986). Meadow bromegrass had a more rapid leaf area index (LAI) development than smooth and hybrid bromegrass following defoliation (see Chapter 4). In the present study, smooth bromegrass increased the surface area of individual leaves more rapidly than meadow and hybrid bromegrass. Thus, rapid reestablishment of leaf area in meadow bromegrass in contrast to the other two bromegrasses did not arise from faster expansion of individual leaves. In other studies, leaf appearance rate tiller^{-1}, which is constant for a given species in relation to growing-degree-days (Cruz and Boval 2000), was greater in smooth bromegrass than meadow

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bromegrass (Lardner et al. 2002). Smooth bromegrass and an experimental hybrid bromegrass population also had greater leaf area tiller^{-1} and number of leaves tiller^{-1} than meadow bromegrass during regrowth (Van Esbroeck et al. 1995). Tiller density following defoliation, however, was greater in meadow bromegrass (see Chapter 5), and this high tiller density compensated for lower individual leaf area. Van Esbroeck et al. (1995) also reported that meadow bromegrass compensated for a relatively small leaf area tiller^{-1} with rapid tiller development and high specific leaf weight.

In the present study, meadow bromegrass had a greater leaf-to-stem ratio and greater regrowth yield than smooth and hybrid bromegrass, implying greater leaf mass, although this trait was not directly measured. Regrowth of smooth bromegrass and an experimental hybrid bromegrass population usually consisted of elongated tillers, and smooth bromegrass stem mass was more than twofold greater than meadow bromegrass in fall regrowth (Baron et al. 2000). Because the leaf blade is the most efficient site for photosynthesis during regrowth (Caldwell 1984), more leaves would increase the potential of photosynthetic assimilation of meadow bromegrass compared to the other two species. In other studies, grasses with rapid regrowth also produced more leaves than sheaths and stems (Detling and Painter 1983; Caldwell et al. 1981). This finding suggests that a higher leaf-to-stem ratio is an important trait for the rapid regrowth of grasses.

Leaf pubescence in desert species reduces the absorption of photosynthetically active radiation more than closely related, non-pubescent species, which dramatically reduced the leaf photosynthetic rate (Ehleringer et al. 1976). In the present study, photosynthetic rate of individual leaves was similar among the three bromegrasses, regardless of leaf pubescence. Even though reflection of radiation is one of the consequences of leaf pubescence (Ehleringer et al.

1976; Yang et al. 2008), this function is more apparent under conditions of high temperature or excessive light intensity (Ehlering et al. 1976; Liakopoulos et al. 2006). In our study, light intensity ranged from 180 to 570 μmol m^{-2}s^{-1}, which is much lower than the light saturation point (approx.1,700 μmol m^{-2}s^{-1}) of C$_3$ grasses. In addition, temperatures during the study were suitable for photosynthesis of C$_3$ species, ranging from 23-25°C. Therefore, light penetration was probably not altered by leaf pubescence for these three bromegrass species. In the present study, the photosynthetic rates ranged 2.5-4.3μmol m^{-2}s^{-1}, which were lower than for other cool-season grasses reported in the literature. For example, the photosynthetic rate of leaf blades in wheatgrass species ranged from 10-20μmol m^{-2}s^{-1} at a saturating light intensity (Nowak and Caldwell 1984). Beside favoral leaf temperature, CO$_2$ level (near 335 ppm) and relative humidity (35-40%) were in the optimum range for leaf photosynthesis of cool-season grasses in this study. Therefore, lower light intensity was likely the factor that caused lower photosynthetic rate in this study.

In C$_3$ species, chloroplast proteins account for about 75% of total leaf N (Chapin et al. 1987). Caldwell et al. (1981) reported that more rapid growth of crested wheatgrass (*Agropyron desertorum* Schult.) than bluebunch wheatgrass (*Agropyron spicatum* Scribn.) was partially attributed to a lower requirement of N per unit area of photosynthetic tissues. Meadow bromegrass has lower N concentrations in the leaves during regrowth than the other two bromegrass species (Baron et al. 2000), which may imply a lower requirement of N to produce leaf tissue.

When a large portion of the foliage was removed by defoliation, the photosynthetic rate in remaining non-defoliated leaves in grasses increased or declined less rapidly with age than those of undefoliated plants (Gifford and Marshall 1973; Detling and Painter 1983). This

enhanced photosynthetic rate has been attributed to increased mesophyll and stomatal conductance in the remaining leaves of the defoliated plant (Gifford and Marshall 1973). This enhanced stomatal conductance may be partially explained by more water being available for defoliated compared to undefoliated plants (Wraith et al. 1987). In the present study, the photosynthetic rate of individual leaves in remaining tillers was similar among the three bromegrasses in the undefoliated control and after partial defoliation. The three bromegrass species were provided adequate soil water during the study. Consequently, soil water availability was not limited in either of our defoliation treatments.

Gross photosynthesis in grasses is largely determined by sward leaf area, being higher in swards with higher leaf area (Parsons et al. 1983). Greater regrowth of meadow bromegrass than the other two bromegrasses also appears to be related to greater leaf area, rather than enhanced photosynthesis after defoliation. This response is consistent with findings of McNaughton (1974) and Detling and Painter (1983) who reported that greater cumulative shoot biomass of grasses results from differences in canopy area rather than variation in net photosynthesis of leaves.

In summary, the two hypotheses tested are rejected. Meadow bromegrass did not differ in photosynthetic rate from the other two species, and its individual leaf area expansion rate was less than that of smooth bromegrass and equal to hybrid bromegrass. The leaf-to-stem ratio was greatest in meadow bromegrass and least in smooth bromegrass following defoliation, suggesting a greater leaf mass in meadow bromegrass.

7.5 Grazing management implications

Regrowth of meadow bromegrass contains more leaves than stems, whereas regrowth of smooth bromegrass has more stem material than leaves, and hybrid bromegrass was

intermediate for this trait. The higher leaf-to-stem ratio of meadow bromegrass likely indicates a

higher forage quality during regrowth, which is important for grazing.

8.0 General Discussion and Conclusions

These studies were designed to provide information on regrowth characteristics of three bromegrasses that are widely used in western Canada for hay or pasture. Meadow and smooth bromegrass are native to Eurasian steppes, where grasses co-evolved with large herbivores. During this co-evolution, grasses developed biological characteristics that helped the plants withstand and recover from heavy defoliation. The hypothesis tested in this study was that meadow bromegrass has superior regrowth ability compared to hybrid and smooth bromegrasses following defoliation because of differences in physiological and morphological characters.

In the present study, regrowth was tested in the field and greenhouse under defoliation at the vegetative and stem elongation stages. With the exception of defoliation at the vegetative stage in the field, meadow bromegrass produced greater regrowth than smooth bromegrass in these two growth environments. Regrowth of hybrid bromegrass was intermediate to smooth and meadow bromegrass. Meadow bromegrass regrows more rapidly than smooth bromegrass and an experimental hybrid bromegrass population in late summer or following frequent defoliation (Knowles et al. 1993; Van Esbroeck et al. 1995; Baron et al. 2000). Hybrid bromegrass has many individual characteristics similar, or intermediate to parental species (Ferdinandez and Coulman 2000), which are associated with the breeding objectives for the development of individual cultivars (Coulman 2004). Characteristics that are the intermediate to the parental species may also explain intermediate regrowth of hybrid bromegrass relative to the other two bromegrasses following defoliation.

Paulsen and Smith (1969) found that smooth bromegrass produces the most yield if plants are defoliated when vegetative and after a recovery period. In the present study, the three bromegrass species produced more regrowth in the field and greenhouse following defoliation at the vegetative stage compared to the stem elongation stage. Regrowth takes place from the elongation of intercalary meristems at the base of existing leaf blades, sheaths, and stem internodes after defoliation at the vegetative stage, while the majority of regrowth comes from axillary buds after defoliation at stem elongation stage (Hyder 1972; Briske 1986). Generally, growth occurs faster from intercalary meristems than from the axillary buds (Cook and Stoddart 1953; Hyder 1972; Briske 1986). Harrison and Romo (1994) suggested that regrowth in smooth bromegrass was not related to a particular growth stage, but rather depends on growing conditions. Environmental conditions following defoliation play an important role in the regrowth of grasses, with temperature and precipitation the main factors (Davidson and Milthorpe 1965). Total GDD during the study years of 2006 and 2007 was higher than the long-term average, with 1704, 1786, and1589 accumulated GDD, respectively. Rainfall from April to June during the study was 26% greater than the long-term average. Therefore, environmental conditions were favorable for regrowth of bromegrasses during the study.

Photosynthetic capacity is known to vary among species (Hikosaka and Shigeno 2009) because of differences in leaf chlorophyll content, stomatal and mesophyll conductance, and leaf photosynthetic N-use efficiency (Field and Mooney 1986; Pons and Westbeek 2004). In the present study, the photosynthetic rate per leaf area was not different among the three bromegrass species (see Chapter 7). In wheatgrasses (*Agropyron*), species with rapid regrowth invested less N per unit leaf area than species with slow regrowth (Caldwell et al. 1981). Meadow bromegrass has lower leaf N concentrations in the leaves during regrowth than the other

two bromegrass species (Baron et al. 2000), which may imply a lower N requirement to produce photosynthetic tissue in meadow bromegrass.

An increase in photosynthetic rates of foliage after partial defoliation has been reported as a mechanism to compensate for defoliation (McNaughton 1979). Following partial defoliation, the rate of photosynthesis declines immediately in the remaining undamaged leaves followed by an increase, or less rapid decline with age than the photosynthetic rate in similar leaves from undefoliated plants (Gifford and Marshall 1973; Detling and Painter 1983; Nowak and Caldwell 1984). In the present study, partial defoliation had no effect on photosynthetic rates in the remaining leaves of undefoliated tillers for all bromegrasses (see Chapter 7), likely due to ideal soil water and temperature conditions in the greenhouse.

Morphological characters, rather than variation in photosynthesis, may play a role in the carbon assimilation after defoliation. In the present study, meadow bromegrass established leaf area most rapidly, while hybrid bromegrass was intermediate and smooth bromegrass was the slowest following defoliation at the vegetative and stem elongation stages (see Chapter 4). In addition, the leaf-to-stem ratio was highest in meadow bromegrass, intermediate in hybrid bromegrass, and lowest in smooth bromegrass (see Chapter 7). The ability to re-establish canopy leaf area is considered the most important characteristic of grasses with rapid regrowth (Caldwell et al. 1981; McNaughton et al. 1983; Briske 1986; Davies 1988). In contrast to canopy leaf area development, the expansion rate of individual leaves was greater in smooth bromegrass than meadow and hybrid bromegrass (see Chapter 7). Similarly, a greater leaf appearance rate tiller[-1] and leaf area tiller[-1] in smooth and hybrid bromegrass than in meadow bromegrass were reported in other studies (Lardner et al. 2002; Van Esbroeck et al. 1995).

In a sward of bromegrass, tiller density was a key factor that determined leaf area development during regrowth (Van Esbroeck et al. 1995; Baron et al. 2000). In the present study, the final tiller density in the field was the greatest in meadow bromegrass, intermediate in hybrid bromegrass, and least in smooth bromegrass regardless of developmental stage of defoliation (see Chapter 5). Percent tiller number increase from the initial counts was the highest in meadow bromegrass (95%), intermediate in hybrid (57%), and least in smooth bromegrass (14%) following defoliation at the vegetative stage, indicating a greater tiller production in meadow bromgrass. Even though tiller density reduced in all three bromegrass species following defoliation at the stem elongation stage, this reduction was less in meadow bromegrass than the other two species (see Chapter 5).

No significant differences in buds tiller $^{-1}$ or elongated buds tiller^{-1} were detected among the three species following defoliation at various stages in greenhouse. Elongation of basal buds into tillers depends on interactions of overall plant vigor, developmental stage, soil nutrients, carbohydrate reserves, environmental conditions, and hormonal regulation (Paulsen and Smith 1969; Langer 1972; Ashmun and Pitelka 1984; Shaver et al. 1986; Murphy and Briske 1992). In the greenhouse study, removal of plants from the field and preparation for potting caused slower tiller growth or even senescence of some tillers in meadow bromegrass, but this was not observed in smooth bromegrass (see Appendix B). In addition, temperatures and light intensity, which are known to affect tiller initiation, differed between the greenhouse and field environments. Thus, elongation of buds into tillers in the greenhouse may not be indicative of tiller responses in the field. A greater number of tillers in a given time period after defoliation at the vegetative stage for meadow bromegrass (see Chapter 5) suggests that more tillers were produced from basal buds in meadow bromegrass in the field.

The carbon required for shoot regrowth immediately after severe defoliation must come from organic reserves in the stem bases of grasses (Reynolds and Smith 1962; Trlica and Cook 1972). For several grasses, the top priority for allocation of stored reserves following defoliation is to produce new leaf material to restore photosynthetic capacity (Donaghy and Fulkerson 1998; Turner et al. 2007). The efficiency of utilizing organic reserves rather than the total concentration of reserves is important to shoot regrowth after defoliation (Richards and Caldwell 1985). The efficiency of utilizing reserves depends on the activity of basal axillary buds and carbohydrate reserves in smooth bromegrass (Reynolds and Smith 1962). In the present study, meadow bromegrass more rapidly remobilized stored reserves to synthesize new shoot tissue than the other two species immediately after defoliation, while the total amount of shoot tissue produced from reserves was similar among the three species after defoliation (see Chapter 6). The efficiency of utilizing reserves for hybrid bromegrass was lower or similar to meadow bromegrass following defoliation at different developmental stages, but was greater than that for smooth bromegrass (see Chapter 6). Therefore, efficient utilization of reserves in the initial phase of regrowth could contribute to more rapid leaf establishment (see Chapter 4) immediately after defoliation, and contribute to the superior regrowth of meadow bromegrass.

Steady-state ^{13}C labeling was used to investigate the use of remobilized and currently assimilated C for leaf and root growth in perennial ryegrass after defoliation. It was estimated that 50% of the carbon was derived from remobilization during the first three days of regrowth, falling to 10% after five days (De Visser et al. 1997). Donaghy and Fulkerson (1997) estimated that remobilization of stored carbohydrate contributed 33%, and current assimilation contributed 66% to regrowth after defoliation. In the present study, it is assumed that the amount of etiolated regrowth represents the maximum possible contribution of organic reserves to normal regrowth

in light. Etiolated regrowth ranged from 10-35% of regrowth in light, which suggests 65-90 % of the C came from photosynthetic assimilation, depending upon the particular developmental stage at the time of defoliation. The dependence of smooth bromegrass on stored reserves was greater than that for the other two bromegrass species (see Chapter 6), which may be related to less residual leaf area or slower development of leaf area following defoliation (see Chapter 4). Even though organic reserves are necessary for the initial phase of shoot growth after defoliation, prolonged dependence on reserves could potentially restrict regrowth of smooth bromegrass because the majority of C needed for regrowth several days after defoliation is derived from photosynthesis rather than stored reserves (Donaghy and Fulkerson 1997; Richards and Caldwell 1985). This greater dependence on reserves could negatively affect regrowth of smooth bromegrass.

N reserves are also an important fraction of total organic reserves and are essential for regrowth (Ourry et al. 1994; Dilz 1966). A ^{15}N study showed that a significant amount of N was remobilized during regrowth in grasses (Phillips et al. 1983; Ourry et al. 1988, 1990). Production of vegetative storage proteins (VSPs) is an example of such N compounds (Tranbarger et al. 1991). In the present study, N concentrations in the stem bases were similar among the three species (see Chapter 6), likely indicating that nitrogen reserves are not a major factor contributing to variation in regrowth among bromegrasses. In addition, plant roots can uptake nitrate and ammonium nitrogen from the soil, which is regulated by the physiological status of the plant and external N concentration (Imsande and Touraine 1994). N required for regrowth of wheatgrasses and cocksfoot is apparently supplied by soil uptake rather than from reserves (Caldwell et al. 1981; Turner et al. 2006).

Severe shoot defoliation often suppresses root development of grasses, which affects water and nutrient uptake (Weaver and Zink 1946; Jameson 1963). Water and N uptake by plants are determined by root distribution and uptake ability of roots (Gastal and Durand 2000). The magnitude of the reduction of root biomass following defoliation was much greater than the reduction in herbage yield in grasses (Johnston 1961). Roots deterioration following clipping was most rapid near the root tips, where root apical meristems and elongation zones are located (Jameson 1963). Root elongation may cease within 24h following a severe defoliation (Crider 1955; Ryle and Powell 1975). Reduction in the number of roots initiated and elongated became progressively more severe with increasing frequency and intensity of defoliation (Crider 1955; Evans 1973). In the present study, below-ground biomass was greatest in meadow bromegrass, intermediate in hybrid bromegrass, and least in smooth bromegrass (see Chapter 4). Greater below-ground biomass in meadow bromegrass suggests a more developed root system that can access soil water and nutrients more efficiently during the regrowth compared to the other two species. In addition, defoliation reduced below-ground biomass of the three bromegrasses compared to undefoliated plants, but the magnitude of the reduction relative to undefoliated plants was greatest in smooth bromegrass (see Chapter 4). Reduction in root growth and productivity are considered to be detrimental to the survival and competitive ability of defoliated plants (Crider 1955; Jameson 1963). This greater reduction of below-ground biomass in smooth bromegrass likely reduced its recovery potential after defoliation.

On the basis of this study, all three bromegrass species can be grazed at the vegetative stage about mid-May in Saskatchewan. Grazing at the vegetative stage will allow livestock early access to high quality forage. Compared to the anthesis stage, bromegrasses have 20% more CP (crude protein) and 18-20% less NDF (neutral detergent fiber) and ADF (acid

detergent fiber) at the vegetative stage (Ferdinandez and Coulman 2001). If bromegrasses are grazed at the stem elongation stage in early June in Saskatchewan, meadow bromegrass and hybrid bromegrass would be the best alternatives. Smooth bromegrass develops leaf area relatively slowly after defoliation at this stage, and its regrowth is less than the other two species. If smooth bromegrass is grazed, a higher defoliation height is recommended to increase residual leaf area to allow more photosynthesis after grazing. In an intensive rotational grazing system, meadow bromegrass can rapidly use organic reserves to re-establish the leaf area, and also develops relatively larger root than the other two species. In addition, meadow bromegrass has more residual leaf area after defoliation because of its basal leaf habit. An adequate rest period, however, is required after defoliation at any stage for persistence and productivity of the three bromegrass species. When defoliated at the stem elongation stage, the rest period should be longer than that at the vegetative stage.

Thus, the hypothesis that meadow bromegrass has greater regrowth ability compared to hybrid and smooth bromegrasses following defoliation because of differences in physiological and morphological characters is accepted. The rapid regrowth of meadow bromegrass following defoliation is attributed to several factors: 1) production and maintenance of a higher tiller density; 2) a higher percentage of tillers remaining in the vegetative stage; 3) rapid LAI development and a higher leaf-to-stem ratio; 4) rapid utilization of organic reserves following defoliation and; 5) relatively greater below-ground biomass. Characteristics that are not associated with rapid regrowth of meadow bromegrass are: 1) photosynthetic rate; 2) nitrogen concentration in the stem bases; 3) total organic reserves and; 4) individual leaf area expansion.

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Appendix A   Regrowth of Two-and Five-Year-Old Stands of Meadow Bromegrass with Varying Percentages of Reproductive Tillers

1. Introduction

Previous research (Ferdinandez 1999) and the present study demonstrated that the percentages of tillers reaching the reproductive stage were different among the three bromegrass species, and a high percentage was assumed to be negatively related to the regrowth of the three species. As a result, an additional experiment was conducted to test if regrowth varies following defoliation in stands of meadow bromegrass with different percentages of tillers reaching the reproductive stage. We tested the hypothesis that a lower percentage of reproductive tillers will be associated with more rapid regrowth. The objective of this study was to determine if percentage of reproductive tillers was associated with regrowth yield of meadow bromegrass stands.

2. Materials and Methods

Sods of Fleet meadow bromegrass (*Bromus riparius* Rehman.) from two different ages of stands (2 and 5 years) were removed from the field in early May 2007. Sods were planted in 20cm pots and placed in the greenhouse. Our assumption was that there would be a lower percentage of reproductive tillers in the older stand of meadow bromegrass based on the findings of Loeppky (1999).

The experiment was a 2 x 3 x 2 factorial randomized-complete-block-design (RCBD) with treatment combinations of two stand ages (two and five-year-old), three defoliation treatments (undefoliated control; defoliation to 5 cm at the vegetative or stem elongation stage) and two soil nutrient levels (with or without 1g pot^{-1} Plant-Prod® 28-14-14 fertilizer). Fertilizer was applied as a solution once at the vegetative stage. Each treatment was replicated four times, and sods were watered when surface became dry. In the greenhouse, light was provided by high intensity sodium lamps with a day length of 16h at 21°C and a night period of 8h at 16°C.

Number of tillers was determined in each pot before the experiment began, and again 20 days after the defoliation. Above-ground biomass was also determined for each treatment. The percentage of reproductive tillers was determined in the undefoliated pots on the same day as the defoliation.

Data were analyzed as a three-way factorial arrangement (stand age X defoliation X soil nutrient) in a randomized-complete-block-design using SAS 9.1.3 Proc Mixed Model (SAS Institute Inc. 2003) to compare regrowth yield and final tiller density of the two meadow bromegrass stands. When ANOVA indicated significant differences (P≤0.05), means were separated using least square means comparison.

3. Results

The percentage of tillers reaching the reproductive stage was 42 and 50% in two-year-old stands with or without fertilizer, respectively, compared to 18 and 14% in the five-year-old stand with or without fertilizer, respectively.

The ANOVA indicated that fertilizer did not affect tiller density; therefore, fertilizer was not considered in the analysis. Final tiller density did not differ between the two stand ages

following defoliation (Table A1). The majority of tillers in the five-year-old stand remained

unelongated and continued their growth after defoliation at the stem elongation stage. The two-

year-old stand had a greater reduction in tiller number because of the higher percentage of tillers

reaching the reproductive stage (elongated tillers), and these tillers died following defoliation.

After defoliation at the vegetative stage or stem elongation stage, final tiller density in the two-

year old stand was reduced from an initial tiller density of 19% for both defoliations, while final

tiller density in the five-year-old stand decreased 5 and 1 % from the initial count, respectively.

Table A1. Tiller density changes of two-year- or five-year-old meadow bromegrass stands after 20 days (420GDD)§ growth in a greenhouse in an undefoliated plant or plants defoliated to 5cm at either the vegetative or stem elongation stages.

Age of stand	Undefoliated control			Defoliated at vegetative stage			Defoliated at stem elongation		
	Initial	Final	%^{y}	Initial	Final	%	Initial	Final	%
Two year	3500	3866 a^{Z}	+10	3500	2833	-19	3967	2733	-19
Five year	2567	3000 b	+17	2633	2500	- 5	2767	2700	-1
P		<0.01			0.37			0.95	
SEM		213			206			206	

Tiller m^{-2}

§ GDD= Growing-degree-days. SEM=standard error of the mean;
^{Z} Means within a column with the same lower case letter are not significantly different (P≤0.05).
^{y} % represents percentage increase (positive value) or decrease (negative value) in numbers of tillers compared to initial tiller count.

Stand age (P=0.002), defoliation (P<0.01) and age x defoliation (P<0.01) effects on

above-ground biomass were significant; however, fertilizer (P=0.13) and its interaction with

stand age and defoliation were not significant. The age x defoliation interaction effect was

further analyzed (Table A2). More above-ground biomass was produced by undefoliated plants

in the two-year-old stand compared to the five-year-old stand. Defoliated plants in two-year- and

five-year-old stands did not differ in above-ground biomass.

Because the five-year-old stand had a lower percentage of reproductive tillers, it was expected that it would produce more regrowth than the two-year-old stand following defoliation; however, tillers were smaller and less vigorous than those in the two-year-old stand. Also, considerable residue accumulated on the surface of the five-year-old stand, which could reduce sod aeration and tiller development. Removal of crop residue was previous shown to increase tiller density in meadow bromgrass (Loeppky and Coulman 2001).

Table A2. Above-ground biomass of two-year- or five-year-old meadow bromegrass stands after 20 days growth in the greenhouse in an undefoliated or following defoliation to 5cm at either the vegetative or stem elongation stage.

Defoliation treatment	Age of stand	Dry matter $(g\ m^{-2})$
Undefoliated control	2	746 a [z]
	5	499 b
Defoliated at vegetative stage	2	180 c
	5	190 c
Defoliated at stem elongation stage	2	180 c
	5	219 c
P value		<0.001
SEM [y]		28

[z] Means within a column with the same lower case letter are not significantly different (P≤0.05).
[y] SEM=standard error of the mean;

4. Summary and conclusions

In the present study, the percentage of tillers reaching the reproductive stage greatly differed between two ages of meadow bromegrass stands; however, final tiller density and regrowth yield were similar between stands. The percentage reduction of tiller density with defoliation was lower in five- than two-year-old stands, which could indicate less impact of defoliation in the five-year-old stand. Greater initial tiller density and larger tillers for plants in the two-year-old stand may have given the two-year-old stand an advantage in regrowth, even with a higher percentage of elongating tillers. Although the different percentages of reproductive

tiller development in these stands provided the opportunity to study the importance of this on regrowth in meadow bromegrass, differential stand age introduced an additional variable, which probably confounded the results. Thus, no conclusion could be drawn.

Appendix B   Comparison of Methodology for Measuring Grass Growth Characteristics in the Greenhouse and Field

1. LAI-2000 leaf area measurement and direct leaf area measurement

Leaf area index (LAI) is one of the most important parameters for assessing the establishment of canopy leaf area. LAI has been calculated as the projected area (one side) of foliage per unit of ground surface (Asner el al. 2007). A direct estimate of LAI can be obtained by harvesting the total leaf biomass in a given area and calculating specific leaf area (Arias 2007). Alternatively, an indirect method of determining LAI is often preferred because such estimates can be completed more rapidly and accurately (Chen et al. 1997). The LAI-2000 plant canopy analyzer is one of the most commonly used optical instruments for the indirect measurement of LAI. LAI is estimated by measuring the amount of diffuse radiation that infiltrates the canopy during the LAI-2000 reading, which estimates a plant canopy index that includes projected stems, inflorescences and leaves (Bolstad and Gower 1990). Therefore, the LAI reading determined by the LAI-2000 should be higher than a direct measurement of LAI, which includes only leaves.

In the present study, a direct estimate of leaf area was made in the undefoliated swards of the three bromegrass species at the reproductive stage to estimate actual leaf area and was compared to the estimate made by the LAI-2000 (Table B1). In the actual LAI measurement, eight 10 x 30 cm areas were sampled for each bromegrass species. All leaf

blades of harvested tillers were separated from the stems for each sample. Direct leaf area was

measured on sub-samples of 50 leaves using LI-3100C Area Meter (Li-Cor, Inc. Nebraska,

USA), then specific leaf area ($cm^2 g^{-1}$) (SLA) was calculated according to its dry weight. Actual

LAI of sample was calculated from the sample dry weight multiplied by SLA. The actual LAI

and LAI-2000 estimation showed a similar ranking of the three bromegrasses, being highest in

smooth bromegrass and lowest in hybrid bromegrass. The actual LAI was lower than LAI-2000

estimates as the latter included all aboveground parts. The LAI-2000 estimates showed the

same ranking of the species as actual LAI determinations, which was adequate for the present

study.

Table B1. Actual leaf area index (LAI) of undefoliated stands of three bromegrass species in the field.

Replication	meadow	smooth	hybrid
1	1.88	1.23	1.20
2	1.56	2.00	1.86
3	1.89	1.80	1.56
4	1.30	2.20	1.66
Means	1.66	1.81	1.57
LAI-2000 analyzer reading	4.24	4.32	4.06

* Tillers were sampled from $0.3m^2$ area for each replication.

2. Plant material preparation

In the present study, a few different experimental units and environments were used

to estimate tiller density and yield responses to defoliation. In the field study, randomly selected

15 x 20cm fixed quadrats were used. For the greenhouse study in 2005, individual tillers were

used to determine tiller development in response to defoliation. Sods of meadow, smooth and

hybrid bromegrass were removed from the field in mid-October 2005. Then tillers at the 2-3 leaf

stages were separated and planted individually in root trainers using a soil mix that contained peat moss, medium grade vermiculite, Scott's "Osmocote Plus" fertilizer (16-8-12) and trace elements. Tiller separation to prepare plants for the experiment disturbed tiller growth of meadow bromegrass more than smooth bromegrass. Because of high tiller mortality and slow growth in meadow bromegrass, the experimental unit was changed in the subsequent greenhouse experiments from individual tillers to a sod of tillers which would be more representative of field growth; however, even in sods, sward cutting to prepare plants for the experiment disturbed meadow bromegrass more than smooth bromegrass, and caused slow tiller growth or even caused some tillers to senescence at the edges of pots. In addition, for smooth bromegrass (species with long rhizomes), spread of rhizomes was limited by the edge of the pot in the greenhouse, which probably could not represent field growth.

In summary, examination of the effect of defoliation on growth in the greenhouse allowed a more constant control of temperature and soil water than in the field; however, the disturbances caused by removing plants from the field and restricted growth in greenhouse pots limited the usefulness of our greenhouse study.

Appendix C

Table C1. Below-ground biomass production of three bromegrass species after 60 days (1260GDD) growth in the greenhouse in undefoliated control or following defoliation to 5 cm at different developmental stages. Data are from two experiments conducted in 2006 and 2007.

Species	Undefoliated control	Defoliated at vegetative stage	Defoliated at stem elongation	Defoliated at vegetative stage + two weeks
		--g m^{-3}---		
meadow	18480 a[y]	14149 ab	10554 bc	7168 c
smooth	16799 a	10949 b	8356 b	5617 b
hybrid	17882 a	12280 b	7840 b	7480 b
P	0.93	0.26	0.28	0.39
SEM[z]	3748	2500	1589	1267

[z] SEM=standard error of the mean; GDD= Growing-degree-days;

[y] Means within a row with the same lower case letter are not significantly different (P≤0.05).

Table C2. Below-ground dry matter of three bromegrass species in an undefoliated control and after 46 days etiolated growth in the field during the summers of 2006 and 2007 at Saskatoon, Saskatchewan.

Species	Undefoliated control	Vegetative stage	Stem elongation stage	Reproductive stage	P	SEM
	---------------------------------- g m^{-3} ----------------------------------					
meadow	7980 a^z E^x	3580 a F	2720 F	3320 F	<0.01	450
smooth	5610 b E	1780 c F	2280 F	2630 F	<0.01	420
hybrid	6430 ab E	2720 b F	2730 F	2770 F	<0.01	610
P	0.03	<0.01	0.64	0.47		
SEM	600	323	182	208		

^{z} Means within a column with the same lower case letter (*a-c*) are not significantly different (P≤0.05).

^{x} Means within a row with the same upper case letter (*E-F*) are not significantly different (P≤0.05).

^{y} SEM=standard error of the mean.